The LDAEP as a Potential Biomarker for Central Serotonergic Activity: Challenges to Overcome

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Contents

Summary

Biological indicators for neurotransmitter activity are of great interest for a better understanding of the pathophysiology of psychiatric disorders. The monoaminergic neurotransmitter serotonin, in particular, plays an important role in the aetiology of many mental disorders. Serotonin has homeostatic effects on brain functioning by regulating the general excitability of neurons, and thus plays a role in modulating perception and behaviour. The loudness dependence of auditory evoked potentials (LDAEP) is a measure of the excitability of neurons in the auditory cortex during the processing of tones of different intensities. Due to a strong serotonergic innervation of the primary auditory cortex it is assumed that serotonin has modulatory effects on these brain areas. A strong LDAEP is thought to reflect a weak serotonergic activity and vice versa.

The LDAEP has been successfully applied in several fields of research. One of its most promising applications is the prediction of treatment responses. However, the LDAEPs validity has been challenged. Particularly, research on some clinical diagnoses is constrained by an inherent heterogeneous symptom constellation within clinical diagnoses based on ICD-10 or DSM-V. It is therefore necessary to assess the symptoms with dimensional measures in order to give evidence to the underlying biological abnormalities. On the other hand, no standardized protocols exist for the application and analysis of the LDAEP. Basically, in order to measure the electrophysiological response of the auditory cortex, dipole source analysis, distributed imaging methods (LORETA) and single electrode estimation have been applied. However, results obtained with different approaches show major inconsistencies.

The aim of the present thesis was to progress in the search of biomarkers suitable for a daily clinical use. So far, findings in schizophrenia research using LDAEP are inconsistent and lack comparability due to the above-mentioned methodological issues. Study I conducted within this thesis investigated patients with schizophrenia by means of LDAEP taking dimensional measures of the symptoms into account. Patients showed higher LDAEP values compared to healthy controls, indicating a lower serotonergic activity. Moreover, predominant negative symptoms were associated with the LDAEP. These findings are in line with other studies, which showed a dysfunctional serotonergic neurotransmission in the genesis of negative symptoms. Study II examined the underlying neural generators during loudness processing using magnetoencephalography (MEG). Magnetic field tomography analysis revealed additional activation of brain regions outside of the auditory cortex. Time course analysis further specified that tones with high intensities were processed within hundreds of milliseconds from the primary auditory cortex as well as the primary somatosensory cortex via the posterior

cingulate cortex into the premotor cortex. These findings have strong implications on the comparability of the different analysis approaches.

In summary, in search of clinical biomarkers it is important to shed light on possible influences of methodological factors in order to improve their reliability and validity. Their application in daily clinical practice would lead to more precise diagnoses and improve treatment strategies by enabling a better prediction of therapeutic outcome.

Zusammenfassung

In der psychiatrischen Forschung sind biologische Indikatoren für die Neurotransmitter-Aktivität im Gehirn von grossem Interesse. Insbesondere Serotonin, ein monoaminer Neurotransmitter, spielt in der Ätiologie vieler psychischer Erkrankungen eine wichtige Rolle. Serotonin wird eine regulierende Funktion im Gehirn zugeschrieben, indem es das Erregungsniveau von Nervenzellen mit der Wahrnehmung und dem Verhalten des Organismus abstimmt. Die Lautstärkeabhängigkeit Akustisch Evozierter Potentiale (LAAEP) ist ein Mass für die Reagibilität der Neurone im auditorischen Kortex bei der Verarbeitung unterschiedlich lauter Töne. Angesichts einer starken serotonergen Innervation des primären auditorischen Kortex wird vermutet, dass Serotonin einen modulierenden Einfluss auf die Aktivität dieses Kortexareals hat. Es wird angenommen, dass eine starke LAAEP eine schwache serotonerge Aktivität wiederspiegelt und vice versa.

Trotz eines beachtlichen Erfolges der LAAEP in verschiedenen Bereichen der neurowissenschaftlichen Forschung in der Psychiatrie, ist dieser Indikator noch nicht genügend validiert, um im klinischen Alltag verwendet werden zu können. Ausserdem sind derartige Biomarker bei Untersuchungen an psychiatrischen Stichproben mit heterogenen Symptomkonstellationen nicht uneingeschränkt anwendbar. Symptome, die unter derselben klinischen Diagnose subsumiert sind, unterliegen möglicherweise unterschiedlichen biologischen Ursachen. Im Kontext solcher Forschung ist es daher erforderlich, dimensionale Beschreibungen der Symptome zu berücksichtigen und mit der LAAEP zu assoziieren. Zum anderen existieren bis anhin keine standardisierten Protokolle für die Applikation und Auswertung der LAAEP. Um Potentialveränderungen im auditorischen Kortex zu erfassen, wurden einerseits Dipolquellenmodelle sowie verteilte Quellenmodelle (LORETA) und andererseits Ableitungen an einzelnen Elektroden vorgenommen. Untersuchungen zeigen, dass die Ergebnisse zwischen diesen Auswertungsansätzen inkohärent sind.

Ziel der vorliegenden Arbeit war es, diesen Limitationen Rechnung zu tragen um die Etablierung der LAAEP voranzubringen. Erkenntnisse zur LAAEP bei schizophrenen Patienten sind uneinheitlich und aus methodischen Gründen schwer miteinander zu vergleichen. In der Studie I wurden Patienten mit Schizophrenie mittels LAAEP und unter Berücksichtigung des Schweregrads unterschiedlicher Symptomcluster, namentlich Negativ- und Positivsymptome, untersucht. Patienten zeigten eine signifikant höhere LAAEP im Vergleich zur gesunden Kontrollgruppe, beziehungsweise eine erniedrigte serotonerge Aktivität. Darüberhinaus war die Ausprägung der Negativsymptomatik stark mit der LAAEP assoziiert. Dies ist in Übereinstimmung mit früherer Literatur, die eine Dysfunktion des serotonergen Systems in der Genese von Negativsymptomen hypothetisierte. In der Studie II

wurden die der LAAEP zugrundeliegenden Generatoren im Gehirn näher untersucht. Mithilfe von Magnetoencephalographie (MEG) und eines verteilten Imaging Verfahrens wurde ermittelt, dass nebst dem auditorischen Kortex auch andere Hirnregionen involviert sind. Eine Analyse der Zeitverläufe ergab eine sequenzielle Verarbeitung von den sensorischen Arealen über den posterioren cingulären Cortex zum Prämotor-Cortex im Millisekundenbereich. Diese zusätzlichen Aktivierungen dürften eine Auswirkung auf die Vergleichbarkeit der unterschiedlichen Auswertungsmethoden haben.

Zusammenfassend ist es von grosser Bedeutung, den Einfluss möglicher methodologischer Faktoren auf die LAAEP zu untersuchen um die Reliabilität und Validität dieses klinischen Biomarkers zu verbessern. Der Einsatz im klinischen Alltag würde eine präzisere Diagnostik und eine bessere Therapieprädiktion begünstigen und so die Behandlung von Patienten massgeblich verbessern.

Abbreviations

5-HT	5-hydroxytrypamine, serotonin
5-HTTLPR	serotonin-transporter-linked promoter region
AEP	auditory evoked potential
AIC	akaike's information criterion
ASF	amplitude-stimulus function
BA	brodmann area
BDNF	brain derived neurotrophic factor
BESA	brain electric source analysis
BET	brain extraction tool
BIC	bayesian information criterion
BRMS	Bech-Rafaelsen melancholia scale
CDSS	Calgary depression rating scale for schizophrenia
COMT	Catechol-O-Methyltransferase
CPZ	chlorpromazine
CSF	cerebrospinal fluid
DAT	dopamine transporter
dB	decibel
DC	direct current
DSA	dipol source analysis
DSM-V	Diagnostic and Statistical Manual of Mental Disorders (5th edition)
EEG	electroencephalography
EPSP	excitatory postsynaptic potential
ERP	event-related potential
FDG	fluorodeoxyglucose
fMRI	functional magnetic resonance imaging
FWE	family-wise error
GABA	gamma-aminobutyric acid
GFP	global field power
GLM	generalized linear models
HAMD	Hamilton depression rating scale
HL	normal hearing threshold
Hz	hertz
ICA	independent component analysis

ICD-10	International Statistical Classification of Diseases (10th revision)
IPSP	inhibiotry postsynaptic potential
ISI	interstimulus interval
LAAEP	Lautstärkeabhängigkeit akustisch evozierter Potentiale
LDAEP	loudness dependence of auditory evoked potentials
LORETA	low resolution electromagnetic tomography
MEG	magnetoencephalography
MFT	magnetic field tomography
MINI	Mini international neuropsychiatry interview
MN	minimum norm
MNI	Montreal Neurological Institute
MRS	magnetic resonance spectroscopy
NIfTI	Neuroimaging Informatics Technology Initiative
NOS	nitric oxide synthase
PAC	primary auditory cortex
PANSS	Positive and Negative Syndrome Scale
PCA	principal component analysis
PCC	posterior cingulate cortex
PET	positron emission tomography
PMC	premotor cortex
pT	pico tesla
RMS	root mean squared
ROI	region of interest
SANS	Scale for Assessment of Negative Symptoms
SERT	serotonin transporter
SL	sensation level; individual's hearing level
SNP	single nucleotide polymorphism
SNR	signal-to-noise ratio
SPECT	single photon emission computed tomography
SPL	sound pressure level
SSRI	selective serotonin reuptake inhibitor
STG	superior temporal gyrus
TFCE	threshold-free cluster enhancement

1. Introduction

1.1 In search of clinical biomarkers in psychiatry

The World Health Organization reported in 2004 that about one person in four will develop one or more mental or behavioural disorders during their lifetime. Mental disorders represent an immense psychological, social and economic burden (Beaglehole & Irwin, 2004). Diagnostic tools in psychiatry have always been based on subjective clinical parameters with high variability that describe symptoms, mental state examinations, and clinical behavioural observations (Hyman, 2007). However, the rising discontent with current diagnostic tools in psychiatry (DSM-V, American Psychiatric Association, 2013; ICD-10, World Health Organization, 1993) refers to the considerable divergency in the aetiology and pathophysiology underlying psychiatric disorders (First, 2010). Patients with the same apparent diagnosis may suffer from a variety of symptoms, conceivably caused by different biological processes, and as a consequence some of them may get treated inappropriately (Linden, 2012). Thus, to improve the quality of diagnoses and the effectiveness of treatment of psychiatric disorders, there is a quest for identifiable biological foundations to psychiatric diagnoses in order to set an objective alternative to the current clinical parameters (Cook, 2008; Leiser, Dunlop, Bowlby, & Devilbiss, 2011; Linden, 2012; Luck et al., 2011; McLoughlin, Makeig, & Tsuang, 2013; I. Singh & Rose, 2009). A promising alternative is provided by bio-markers, which have been defined as having the 'characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention' (Biomarkers Definitions Working Group, 2001, p. 91). In other words, in the context of psychiatric disorders, an ideal biological marker presents high diagnostic specificity and sensitivity and is qualified for prognosticating the course of an illness (including the detection of individuals at-risk) and for predicting and monitoring response to treatment (I. Singh & Rose, 2009). These efforts will be useful for guiding tailored individualized therapy in clinical practice.

Several potential biomarkers have been proposed in psychiatric research, for example specific molecules and metabolites in cerebrospinal fluid (CSF) and urine, sleep states, and physiochemical responses (Cook, 2008). Currently, however, genetic testing and neuroimaging are the main techniques for identifying biomarkers in psychiatry (I. Singh & Rose, 2009). Within neuroscience, neurobiological, neuroanatomical or neurophysiological findings can define an imaging biomarker (Linden, 2012), for instance [F-18]-fluorodeoxyglucose (FDG) positron emission tomography (PET) has been used in alzheimer research (Herholz et al., 2002), cortical grey matter density measures were proposed in psychosis (Sun et al., 2009), and stages of sleep/wakefulness characterized with

electroencephalography (EEG) in depression (Steiger & Kimura, 2010). In the context of a neuroscientific approach to defineing a biomarker it is critical that the measure is independent of individual influences by the observer or environmental influences, as well as the thoughts and attitudes of the subject (Kraemer, Schultz, & Arndt, 2002). Furthermore, the concept of endophenotypes has gained great attention in unravelling significant heterogeneity which is intrinsic to some psychiatric disorders (Tandon, Nasrallah, & Keshavan, 2009). Endophenotypes are disease-associated neurobiological correlates that represent intermediate traits in the causal connection between genotypes and phenotypes[1], whereby the main advantage is that they are exposed to less complex genetic determination than clinical phenotypes (Gottesman & Gould, 2003; Hyman, 2007; Kawohl & Hoff, 2010). Moreover, the dysfunctional correlates are heritable traits that occur even in unaffected relatives of patients and help to identify the genetic factors underlying susceptibility to mental illnesses.

However, numerous challenges exist that researchers and clinicians are confronted with in the quest for sensitive and reliable imaging biomarkers in psychiatry (Linden, 2012; Luck et al., 2011; I. Singh & Rose, 2009). The above-mentioned problem of heterogeneity of symptoms within a given clinical diagnosis provides on the one hand the motivation to promote biomarker research, and on the other hand creates a serious pitfall in applying neuroimaging biomarkers. Referring to the latter, this is mainly because biomarkers are still related to diagnoses based on the DSM-V or ICD-10 that underly complex pathogenic biological processes. A possible solution to overcome the diagnostic uncertainties of phenotypic definition is the use of dimensional measures of mental functions and their underlying neural circuits to classify disorders (Insel et al., 2010; Miller, 2010). The main goal in biomarker research is to move beyond current diagnostic definitions of psychopathologies by identifying the underlying processes of significant cognitive, affective and social processes that are associated with particular mental disorders (Hyman, 2007). Furthermore, in order to be widely useful as biomarkers, measures require the power to distinguish patients with a particular psychiatric disorder both from controls and from patients with different disorders, and at best to characterize individuals within a group (Linden, 2012). However, their sensitivity, specificity and ability to make predictions should be specified (Cook, 2008).

A complete understanding of the psychometric properties (i.e. quantifiable attributes as for example validity and reliability that relate to the potency of the measurement) of a biological marker is

[1] Phenotypes are attributable to observable characteristics of traits that are produced by the interaction of genetic expression and environmental influences (Birbaumer & Schmidt, 2005).

therefore required (Luck et al., 2011). The biomarker's validity and reliability must be examined in nonclinical samples before it can be implemented in routine clinical practice. Reliability is a measure of the precision of scientific measurements and the extent to which they reflect the true variance of a signal not contaminated by random error (Hensch, Herold, Diers, Armbruster, & Brocke, 2008; Schmidt, Le, & Ilies, 2003). That means that optimally re-testing under the same conditions should lead to identical results, reflecting high consistency on each repetition. The evaluation of the validity of a biomarker is more complex and requires sufficient reliability. Essentially, construct validity states the degree to which a biomarker truly tests what it intends to test (i.e. the accuracy of the measurement) and to what extent it can explain the underlying biological phenomenon (Clark & Watson, 1995).

Considering all these caveats, standardized acquisition and analysis procedures should be elaborated upon to assure quality. The great variability of the biomarkers' corresponding parameters (for some examples see chapter 2.2) that is inherent in most neuroimaging approaches may limit the comparability of findings and conceivably weaken the validity of the biomarker.

Furthermore, for successful implementation in daily clinical use, additional consideration has to be given to cost and ease of the pipeline of the biomarker handling. EEG is a powerful tool that does not need expensive equipment and is relatively easy to apply in psychiatric institutions with no access to high-end neuroimaging laboratories (McLoughlin et al., 2013). EEG is particularly applicative in large-sample studies, which are generally required in genetic imaging. Moreover, the non-invasive nature of EEG allows for a relatively comfortable measurement of children and anxious or suspicious patients who are not suitable to undergo noisy, highly motion-susceptible (functional magnetic resonance imaging; fMRI) or invasive (PET) neuroimaging measurements (McLoughlin et al., 2013). EEG is generally easy to translate from animal models to human, as the underlying neural processes that are directly measured through EEG are similar between species (Woodman, 2012). The straightforwardness of pharmacological manipulations and genetic modification in rodents and primates can help to assess EEG measures as a potential biomarker (Luck et al., 2011).

The aim of this dissertation is to shed light on the loudness dependence of auditory evoked potential (LDAEP), a potential EEG-based biomarker proposed to reflect neurotransmitter activity in the brain. Pertaining to the challenges for biomarkers that have to be overcome before they can be implemented in clinical practice, as described above, this work refers to specific problems regarding the LDAEP as a biomarker.

First, the concept of the LDAEP is elaborated (chapter 2.1), followed by an overview of current methodological variables that are applied when setting up an experiment with LDAEP (chapter 2.2). In addition, a description of what is currently known about the underlying sources involved in

LDAEP (chapter 2.3) is given. Furthermore, in the example of schizophrenia and LDAEP, the problem of relying on diagnostic tools in research is illustrated (chapter 2.4). In chapter 3 the main aims of this dissertation are summarized, followed by a short description of the methods applied in the experiments (chapter 4). Given the tremendous heterogeneity that characterizes schizophrenia and its related difficulty in the validation of the biomarker, study I (chapter 5.1) addresses the heterogeneous phenotypes within schizophrenia by studying the biological correlates within symptom clusters of this disorder, namely negative and positive symptoms. With the objective of improving the reliability and validity of the LDAEP, study II identifies the neural correlates of intensity perception (chapter 5.2). These findings help to better understand which processes are involved during the processing of LDAEP and therefore improve the validity of this potential biomarker. Additionally, the findings provide suggestions for standardized analytical methods, which will increase the reliability of the LDAEP. Chapter 6 highlights the main limitations and conclusions that can be drawn from these studies.

2. Theoretical background

2.1 Loudness dependence of the auditory evoked potential (LDAEP)

2.1.1 Parameterization of the LDAEP

The loudness dependence of auditory evoked potentials (LDAEP) represents an increase in the neuronal response in the auditory cortices with an increase in sound level (Juckel, 2005). This is reflected in a variation in amplitude (Fig. 1, panel B) that is considered to show the amount of reactivity of the generating cortical neuronal networks in relation to the intensity of the presented tones. This reactivity is dependent on the organism's protection mechanism from sensory overstimulation (Buchsbaum & Silverman, 1968; Hegerl & Juckel, 1993). The terminology to describe the LDAEP varies in the extant literature. Some authors use the term IAEP for intensity dependence of the auditory evoked potentials (e.g. Hensch et al., 2006). However, the term LDAEP is most widely used (e.g. Gallinat et al., 2007; Juckel, Gudlowski, et al., 2008; Ostermann, Uhl, Köhler, Juckel, & Norra, 2012; Park, 2014). Notably, the activation upon auditory processing reflects the subjective perception (loudness) rather than a physical entity (intensity) (Uppenkamp & Roehl, 2013). In this thesis the term LDAEP is therefore used, as in previous publications of our group. In the 60s and 70s the observed phenomenon of augmenting/reducing was the focus of attention, precursory to the LDAEP paradigm. In their concept of augmenting/reducing, Buchsbaum and Silverman (1968) hypothesized that the individual's enduring mode of 'stimulus intensity control' can be assigned into two classes: augmenters show increased responses to increasing stimuli, whereas reducers show reduced responses. However, methodological criticism (e.g. intensity site, electrode recording and stimulus modality) have led to a reformulation of the concept of augmenting/reducing (Carrillo-de-la-Pena, 1992; Connolly & Gruzelier, 1982) and in its place the LDAEP paradigm moved up (Hegerl & Juckel, 1993). The pioniering idea was to determine the loudness intensity effect directly for the auditory cortex through the use of dipole source analysis (Hegerl, Gallinat, & Mrowinski, 1994). Moreover, due to advanced parameterization of the LDAEP, the dichotomy of the former concept was replaced by a continuous variable for which any value is possible within a range. Such a variable generally shows improved reliability.

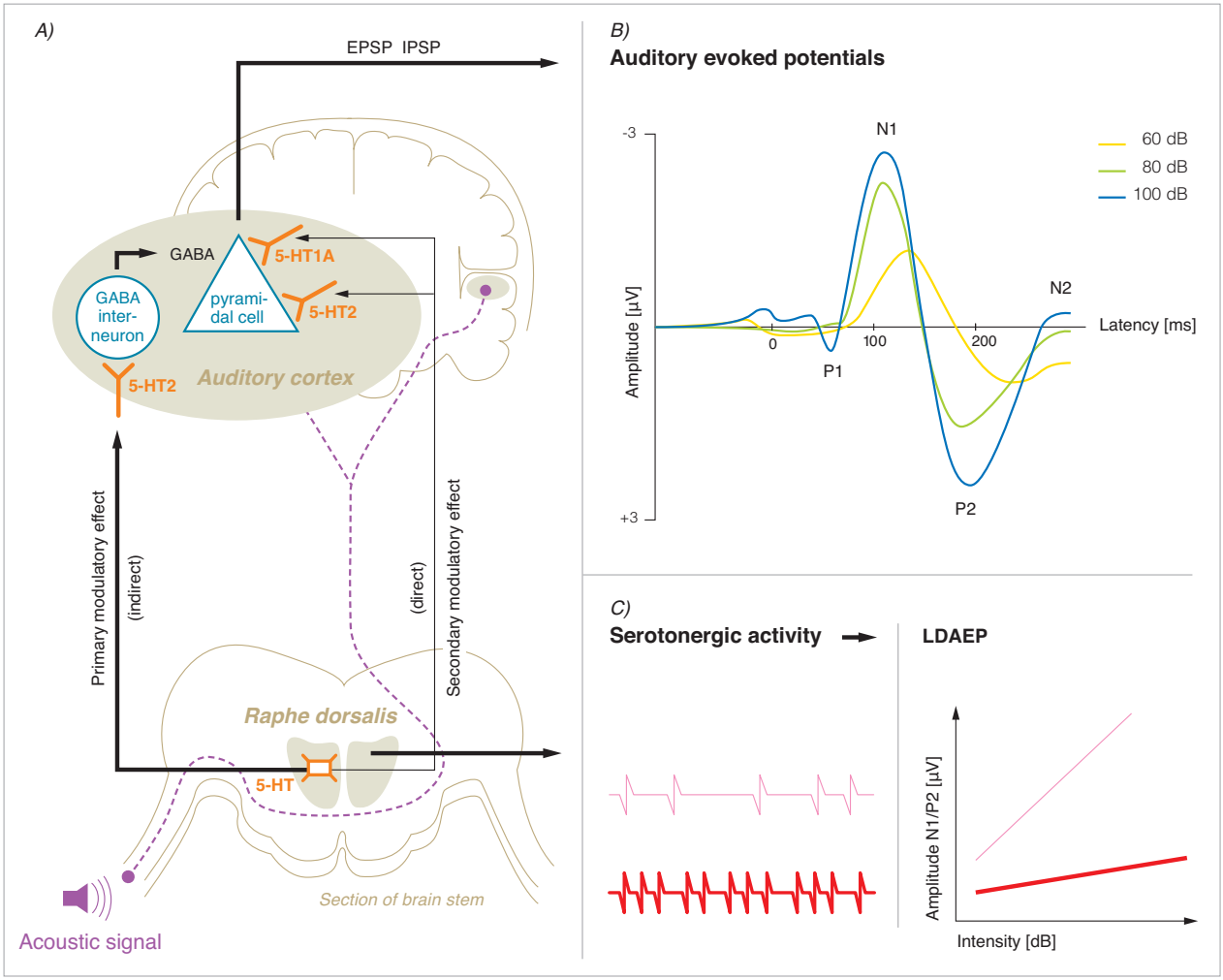

Fig. 1.

A) Neurobiological model of serotonergic modulation of the loudness dependence of auditory evoked potentials (LDAEP) in the primary auditory cortex (PAC). The PAC is highly innervated by serotonergic (5-HT) neurons that are located in the raphe nucleus situated in the reticular formation of the brain stem. Serotonergic activity from the raphe dorsalis has a modulatory effect on the PAC, mainly via the primary modulatory pathway. The 5-HT inhibits indirectly, i.e. via GABAergic interneurons the pyramidal cells of the PAC. Alternatively, there is a direct modulatory effect on the pyramidal cells both via 5-HT_2 (excitatory) and 5-HT_{1A} (inhibitory) presynaptic receptors. This secondary pathway allows for the fine-tuning of the 5-HT influence. Consequentially, these 5-HT modulatory effects prime the reagibility of the pyramidal cells during the processing of an acoustic signal and in turn influence the excitatory- and inhibitory postsynaptic potentials (EPSP/IPSP). Adapted from *Serotonin und akustisch evozierte Potentiale* (p. 147), by G. Juckel, 2005, Darmstadt: Steinkopff Verlag. Copyright 2005 by Steinkopff Verlag Darmstadt. Adapted with permission.

B) Example of loudness dependence of auditory evoked activity following auditory stimulation with different sound pressure levels of 60, 80, and 100 dB SPL (sound pressure level). The N1/P2 amplitude at Cz increases with rising sound level.

C) The inverse relationship between 5-HT activity and LDAEP is illustrated. A high firing rate of the serotonergic neurons in the raphe nuclei results in a weak LDAEP (i.e. a small increase in N1/P2 amplitude with increasing intensity, illustrated in bold type) and a low firing rate results in a strong LDAEP (i.e. a large increase in N1/P2 amplitude, illustrated in light type). The LDAEP slope can also be respectively interpreted as weak or strong reagibility of the PAC in accordance with panel A.

Ever since, the LDAEP has been investigated in a large number of studies of humans and animals (for some examples refer to 2.1.2). However, great variability exists in the auditory evoked potential (AEP)-components used to calculate the LDAEP on the one hand and in the calculation of the amplitude-stimulus intensity function (ASF) itself on the other hand. Regarding the former, Hegerl and Juckel (1993) originally focused on the N1/P2 peak-to-peak amplitude because of its high reliability in comparison to N1 or P2 (Beauducel, Debener, Brocke, & Kayser, 2000) and because of its lower sensitivity to latency shifts (Connolly & Gruzelier, 1982) (different components are illustrated in Fig. 1, panel B). The N1/P2 magnitude can be calculated in several different ways: effective amplitude, peak-to-peak amplitude or as the root mean squared effective amplitude over the epoch of the Nl/P2-component (for a definition see Scherg & Von Cramon, 1990). Referring to the latter, the slope of the ASF function can be calculated either as the line of best fit, by using the least-square technique (linear slope), or from the median of all possible lines between any two intensities (median slope) (Hegerl & Juckel, 1993). The median slope is presumed to better cope with the extreme values of intensity (Connolly & Gruzelier, 1982). Overall, the steeper the line, the higher the degree of loudness dependency, irrespective of which slope is used.

Moreover, the LDAEP occurs with high inter-individual (Buchsbaum, 1971) and low intra-individual variability (Carrillo-de-la-Pena, 2001; Hegerl et al., 1994; Hensch et al., 2008). A genetic background is thought to play an important role, as similiarity of LDAEP within family is far more pronounced than between genetically unrelated pairs (Sándor, Áfra, Proietti-Cecchini, Albert, & Schoenen, 1999).

2.1.2 A biomarker for central serotonergic activity

A growing body of evidence from human and animal studies suggests that the physiological properties of the auditory cortex in relation to intensity variation of a tone are directly associated with the serotonin (5-HT; 5-hydroxytrypamine) released at the synapses in the primary auditory cortex (PAC). An inverse relationship is assumed, whereby a weak LDAEP indicates high serotonergic activity, and vice versa (Fig. 1, panel C). For a detailed description of the serotonin modulation theory please refer to Fig. 1, panel A and C and to Hegerl et al. (1994); Hegerl and Juckel (1993).

The serotonin system plays an important role in the pathophysiology of major psychiatric disorders such as depression, schizophrenia, anxiety disorders, posttraumatic stress disorder, chronic pain syndrome and substance related disorders, and thus is a target of pharmacotherapeutic interventions (Hegerl & Juckel, 2000). Reliable indicators of this system are urgently needed for clinical and scientific interest. Peripheral measurements of precursors and metabolites of serotonin in serum and CSF only reflect the brains serotonergic activity indirectly and their relevance to functional aspects

of the neurotransmitter system remain unclear (Bell, Abrams, & Nutt, 2001; Murphy, 1990). There is no gold standard for measuring brain serotonergic activity directly in humans. However, a strong evidence exists for the serotonin hypothesis and the LDAEP owing to data from animal studies (O'Neill, Croft, & Nathan, 2008).

Several studies in mammals used epidural (i.e. for intraoperative monitoring of evoked potentials) electrode placement while challenging the serotonergic system (Juckel, Hegerl, Molnar, Csepe, & Karmos, 1999; Juckel, Molnar, Hegerl, Csepe, & Karmos, 1997; Manjarrez, Hernandez, Robles, & Hernandez, 2005; Wutzler et al., 2008). These studies show an inverse relationship between the serotonergic system and evoked potentials in the PAC by intravenous administration or direct microinjection into the dorsal raphe nucleus of 5-HT agonists and antagonists. Importantly, the neural pathways involved in the processing of loudness intensity variation in animals share anatomical and functional similarities to humans and thus the results from these studies are translatable (Juckel, Csépe, Molnár, Hegerl, & Karmos, 1996).

Investigations on patients suffering from psychiatric disorders that underlie aberrant serotonergic neurotransmission, genetic polymorphisms of serotonin and direct pharmacological challenge studies in humans all serve to strengthen the assumption of an inverse relationship between LDAEP and serotonin (consider O'Neill, Croft, et al., 2008 and Park, Lee, Kim, & Bae, 2010 for a detailed review).

Most promising are studies that predict the treatment response to specific pharmacological interventions. LDAEP values that reflect the level of serotonergic activity are collected prior to treatment and, in turn, indicate whether the patient would be a good or bad responder to treatment. In affective disorders strong LDAEP (i.e. low serotonergic level) is proposed to reflect a favourable response to selective serotonin reuptake inhibitors (SSRI) and preventive lithium treatment (Hegerl, Gallinat, & Juckel, 2001; Leuchter, Cook, Hunter, & Korb, 2009; Park & Lee, 2013).

Even though these studies are encouraging, particularly in improving antidepressant medication, the effect on the LDAEP after SSRI treatment is contradictory (Gallinat et al., 2000; Guille et al., 2008; Juckel et al., 2003; Linka, Sartory, Wiltfang, & Mueller, 2009; Norra et al., 2008; Oliva et al., 2010; Segrave, Croft, Illic, Phan, & Nathan, 2006; Simmons, Nathan, Berger, & Allen, 2011; Uhl et al., 2006). Simmons, Nathan, Berger, & Allen (2011) and O'Neill et al. (2008) argue that a chronic manipulation of the serotonergic system might have a stronger impact on the LDAEP than acute changes. This statement is based on the fact that acute manipulations of the serotonergic system have often failed to change the LDAEP (Guille et al., 2008; Norra et al., 2008; Oliva et al., 2010; Uhl et al., 2006) and variables representing a trait, such as genotypes (Hensch et al., 2006), vulnerability to

psychiatric disorders (Gudlowski et al., 2009) or characteristics of long-term ecstasy-users (Wan, Baldridge, Colby, & Stanford, 2009), show stronger associations with the LDAEP.

The identification of individuals at risk for psychiatric disorders (or at least before full-blown illness) is of current interest. Early detection allows for early intervention, which is of high importance, as timely interventions could prevent the manifestation of the illness. Many patients with affective disorders experience recurrent thoughts of death and hopelessness that are associated with an elevated risk of suicide attempts. For clinicians, it would be most helpful to have a measure that reliably predicts which individual will show suicidal behaviour and which will not. It is hypothesized that a strong LDAEP, indicating low serotonergic activity, is associated with acute suicidal ideation, alongside a history of suicide attempts (T. J. Chen et al., 2005; D.-H. Kim & Park, 2013; Park & Lee, 2014). A closer investigation into the process underlying suicidal attempts reveals that directly after a suicide attempt, high fluctuations of serotonergic levels are apparent, beginning at higher levels and falling to lower levels about one week later (Uhl et al., 2012). Another crucial observation is that individuals at risk for psychosis with characteristic prodromal symptoms already exhibit enhanced serotonin levels, measured by means of LDAEP, before the onset of psychosis (Gudlowski et al., 2009). In the further course of the illness the aberrant serotonergic level remains high (Juckel, Gudlowski, et al., 2008). This substantiates a strong rationale of LDAEP being a marker for vulnerability rather than for expression of illness itself. Altogether, the results in studies into prediction of progression of suicidal ideations potentially leading to suicidal behaviour and early detection of psychotic disorders lead to the hypothesis that dysregulated serotonergic neurotransmission reflects a trait marker of these illnesses that is already present before the onset of the disease or behaviour. In this manner the LDAEP holds promising characteristics for use as a biomarker (Luck et al., 2011). Psychiatric disorders underlie very complex etiological and pathogenetical mechanisms, though it will be very difficult to identify the risk by a single measure. A model combining genetic and biological features may be developed to refine the predictability of this risk.

LDAEP is a promising endophenotype in human serotonin research with the potential to associate the causal pathway from genetic variation to the phenotypes (Hensch et al., 2006). Several psychiatric disorders that are characterized by the presence of a dysfunctional serotonergic activity are linked to single nucleotide polymorphisms (SNPs) of the serotonergic system (Strobel et al., 2003) and therefore a potential association between LDAEP and genetic variants of the serotonergic system is of great interest. So far, SNPs of the 5-HT_{1B} receptor (Juckel, Hegerl, et al., 2008) and the serotonin-transporter-linked promoter region (5-HTTLPR) (e.g. Gallinat et al., 2007; Hensch et al., 2006; Juckel, Hegerl, et al., 2007; Strobel et al., 2003) have been associated with the LDAEP, but with contradictory findings. This is probably due to limited effect sizes and methodological variations (Hitz, Wyss, Hengartner, & Kawohl, in preparation). However, further candidate genes, such as the

Catechol-O-Methyltransferase (*COMT)*-gene (Juckel, Kawohl, et al., 2008), the nitric oxide synthase (*NOS1, NOS3*)-genes (Kawohl, Giegling, et al., 2008) that are linked with the dopaminergic system and the brain derived neurotrophic factor (*BDNF*)-gene (Juckel et al., 2010) have been investigated in association with the LDAEP. These genetic studies point out that the LDAEP may also underlie influences by other changes in neurotransmission. For further discussion of this issue refer to chapter 6.3.

2.2 Overview of methodologies used in LDAEP research

The variety of the methodologies applied in LDAEP research is manifold. This limits the comparability of the stated results across the field and leads to inconsistent findings. Furthermore, to be applied as a biomarker in future clinical use, it is mandatory to define optimal parameters best used in the implementation and analysis of LDAEP studies, thereby enhancing the reliability of the LDAEP. Variety in the LDAEP methodology emerges with respect to stimulus properties, demographic and habitual characteristics, and parameters of LDAEP. The following section gives an overview of potential pitfalls to consider when planning an experiment.

In terms of **stimulus properties**, differences between studies can be mainly observed in the different use of stimulus intensity, initial intensity level, number of intensity levels, interstimulus interval (ISI), order of stimuli presentation and frequency of the tones. Beauducel et al. (2000) investigated the reliability of the LDAEP and proposed that stimulus intensity should vary between 60 and 95 dB sound pressure level (SPL) with at least five different intensity levels. However, the range of intensities used in LDAEP studies varies between two and six different intensities. Another group found increased reliability with higher intensity levels, but they pointed out that it is unclear whether the results would remain stable if one high intense tone were to be repeatedly presented or the same tone embedded in a range of other intensities (Hensch et al., 2008). The optimal sweeps, i.e. averaged epoches per intensity, should be around 100 to achieve sufficient signal-to-noise ratio (SNR), but time-on-task effects such as increasing slowdown, habituation, boredom and fatigue must be considered and could vary between patient samples (Hensch et al., 2008). For a more detailed description of EEG methodology please refer to chapter 4.1. There is evidence that the initial intensity level (30 or 70 dB SPL) affects the growth in N1 amplitude with increasing intensity (Uhl et al., 2011). Moreover, the reference level of the presented stimuli varies in LDAEP studies and individual's hearing thresholds are mostly disregarded. Besides the most commonly used physical reference, referred to as sound pressure level (SPL), there exist audiometric references relative to normal hearing threshold (HL) or to the individual's hearing level (SL) (Picton et al., 2000). Some studies reported that when the stimulation rate is increased (ISI>4s), further generators, presumably in modality unspecific areas underlying the N1 component, are represented in the recorded waveform

(Budd, Barry, Gordon, Rennie, & Michie, 1998; Hari, Kaila, Katila, Tuomisto, & Varpula, 1982; Näätänen & Picton, 1987). These additional sources could contribute to the scalp derived potentials and may mislead interpretation of what is measured (for further information refer to chapter 5.2). Furthermore, it must be considered that the order of presentation of the tones (randomised, pseudo-randomised or in a block) may have an influence on the subject's perception, for instance by enhancing attention to a specific tone that is out of range and this may lead to a pronounced response (Carrillo-de-la-Pena, 1999; Zacharias, Konig, & Heil, 2012). Soeta and Nakagawa (2001; 2011) investigated the effects of different stimulus frequencies (250 Hz, 1000 Hz, >1000 Hz) on the loudness dependent slope of a continuous tone (1000 ms) and reported a lower increase in amplitude with higher frequencies.

Regarding **demographic and habitual characteristics**, most studies on the LDAEP included both men and women in the sample and gender was not considered a confounding influence. However, a gender effect has been observed in recent studies indicating a stronger LDAEP in healthy females than males (Oliva et al., 2011), which is in line with findings of reduced mean rates of serotonin synthesis in females (Nishizawa et al., 1997). Hormonal fluctuations across the menstrual cycle or due to contraceptive use may have additional influences on auditory evoked potentials (Walpurger, Pietrowsky, Kirschbaum, & Wolf, 2004). These results are in line with the findings of another study, where lower variances in the LDAEP values were reported for men (Hensch et al., 2008). Another factor that has to be accounted for is nicotine use, as it was demonstrated to have an effect on sensory response in mice (Metzger, Maxwell, Liang, & Siegel, 2007) and also on the LDAEP (Gallinat et al., 2005). However, nicotine use was not associated with genetic traits and is thought rather to be a state dependent factor.

The parameterization of the LDAEP varies highly between the studies. On the one hand, there are several ways to calculate the ASF as well as to choose the AEP-components (for a description of the different possibilities of doing so, refer to chapter 2.1.1). On the other hand, some studies reported scalp potentials from mostly midline electrodes (Fz, Cz), whereas others implemented source analysis of the PAC. In the following section these analysis methodologies are shortly summarized with regard to reliabilities.

In single electrode estimation, features such as amplitude or latency are analysed only at electrode channels of interest or channels at which the feature is maximally expressed. This method is most commonly used in LDAEP research, which might be due to its ease of use, as single electrode estimation is the simplest and most rapidly completed procedure (Carrillo-de-la-Pena, 1999; Hensch et al., 2008).

The problem which occurs with scalp-recorded data is that the true brain sources are unknown and only a mixture of activity from different generators including non-cerebral (e.g muscle or ocular) artefacts is represented on the scalp (McLoughlin et al., 2013). Hensch et al. (2008) focussed on the reliability of these parameters in LDAEP and reported satisfying retest, odd-even and split-half reliabilities after three weeks for Cz, C3 and C4 electrodes (r = .60-.90). The highest reliabilites were reported at Cz with linear slopes (females r = .88, males r = .82). Accordingly, using principal component analysis (PCA) to accurately identify the independent event-related potentials (ERP) components, the N1/P2 slopes and the P2 slope compared to P1, N1 and P1/N1 slopes reached high temporal stability (Beauducel et al., 2000). These findings are in line with reliability measurements over one year that reached good reliabilities (r = .60-.80) for the N1/P2 slopes at Fz and Cz (Carrillo-de-la-Pena, 2001). What makes comparisons difficult is that studies in LDAEP research evaluated different components, namely P1, N1, or P1-P2 (e.g. Gudlowski et al., 2009; Linka, Sartory, Gastpar, Scherbaum, & Müller, 2009).

The initial theory of LDAEP by Hegerl and Juckel (1993) suggests that the ideal methodological assessment strategy is to assess the loudness dependence in the PAC (for a detailed argumentation see Hegerl et al, 1994) . Comparable to scalp channel measures, the retest-reliabilities (after three weeks and one year) of N1/P2 slopes for dipoles obtained with brain electric source analysis (BESA) are excellent, especially for tangential dipoles (r = .88), which are thought to reflect activity in the PAC. Individual differences in skull thickness or cortical folding may account for the variability of the brain signal measured at the scalp. Thus source localisation could be used to counteract these influences (Luck et al., 2011). Source analysis techniques such as BESA and low resolution electro-magnetic tomography (LORETA) are ideal tools for this purpose (for a description see chapter 4.2).

2.3 Underlying sources in loudness perception

In the quest for an informative biomarker, it is inevitable to improve the validity of the candidate marker. In order to specify whether the LDAEP measures what it claims to measure, the underlying mechanisms must be elucidated and integrated into existing knowledge about sensory processing and cognitive functions. Especially when analysing single electrode sites or setting dipoles to represent the brain sources a priori, extensive knowledge of the brain regions which are involved in loudness perception is necessary. Additionally, the generators activated may depend on the context the stimuli are presented in (e.g. ISI, intensity level) (see chapter 2.2). The involvement of the supratemporal plane in the generation of LDAEP as proven by EEG, fMRI and magentoecenphalography (MEG) is beyond controversy (Jäncke, Shah, Posse, Grosse-Ryuken, & Müller-Gärtner, 1998; Mulert et al., 2005; Neukirch et al., 2002; Wood et al., 1984). Moreover, it has been reported that subcortical areas

are involved in auditory processing and show sensitivity to different intensity levels (W. T. Roth, Horvath, Pfefferbaum, & Kopell, 1980). Although the N1 and P2 components are exogenous components (thought to be modulated only by physical characteristics of the stimulus) an unspecific component is assumed to contribute to the measured signal. It is ambiguous whether this unspecific component is related to higher cognitive functions such as orientation of attention linked to the execution of motor response (Hari et al., 1982; Näätänen & Picton, 1987).

2.4 Schizophrenia: Uncertainties about phenotypic definition in psychiatry

Schizophrenia is a chronic mental illness characterized by disturbances in thought, perception, affect, behavior, and communication. About one percent of the world's population is affected during their lifetime (Perala et al., 2007) and their symptoms often lead to impaired functional outcome, resulting in poor success in social and professional life (Rössler, Salize, van Os, & Riecher-Rössler, 2005). The current diagnostic system (DSM-V; American Psychiatric Association, 2013) contains a broad spectrum of symptoms required for schizophrenia, which can be roughly categorized as positive and negative symptoms (Andreasen et al., 1994). Positive symptoms are core features of the illness and include hallucinations, delusions and formal thought disturbances. They are most often accompanied by negative symptoms that indicate a deficit in normal mental functions and consist of affective flattening, alogia (impoverishment of the language), avoliation (reduced emotional expression), anhedonia (inability to experience pleasure) and attentional impairment (Andreasen & Black, 2001). Supposedly, negative symptoms have a more stable developmental course than positive symptoms and appear to be present at the prodromal as well as the residual state of the disease (Davidson & McGlashan, 1997). Primary negative symptoms are directly related to the disease process itself and secondary negative symptoms occur as a result of the illness or because of medication side effects (Kirkpatrick, Fenton, Carpenter Jr., & Marder, 2006). Importantly, with contemporary measurements of psychopathology, it is not possible to distinguish primary from secondary negative symptoms (Lindenmayer, Harvey, Khan, & Kirkpatrick, 2007).

It is still a matter of debate whether the current diagnostic criteria for schizophrenia are useful for neuroscientific research (Hyman, 2007). First, the inherently heterogeneous symptom presentation of schizophrenia leads to different symptom constellations subsumed under the same diagnosis, hence the biological processes may also differ substantially. Second, the traditional diagnostic system only allows categorizing of the disorders in entities being either ill or healthy, not allowing for the characterization of gradations of disease severity within a category (McLoughlin et al., 2013). A wide community of researchers propose a dimensional approach based on symptom clusters to provide a better perspective on phenomenology of psychopathology, because it considers symptom

patterns that share neurobiological, genetic and developmental features (e.g. negative symptoms or disorganized speech) (Barch et al., 2013). In schizophrenia research, however, some symptoms, namely delusions and hallucinations have been observed in a continously distributed fashion in the general population (Rössler et al., 2007). Nonetheless, in the currently released 5th edition of the DSM, a dimensional approach was not considered (American Psychiatric Association, 2013).

Concomittant to these shortcomings, it is not surprising that the exact pathophysiology of schizophrenia still remains fragmented (Tandon, Keshavan, & Nasrallah, 2008). The dopamine model is the predominant approach to explaining the prevalent symptoms as an imbalance of dopaminergic neurotransmission. It is supposed that an excess activation of subcortical dopamine D_2-receptors is responsible for positive symptoms, whereas a hypoactivation of cortical dopamine D_1-receptors leads to negative and cognitive symptoms (K. L. Davis, Kahn, Ko, & Davidson, 1991; Knable & Weinberger, 1997). The fact that atypical neuroleptics develop their pharmacologic effects via the dopaminergic and serotonergic system likewise, lead to the extension of the dopamine hypothesis (Meltzer, 1999). Serotonin ought to reinforce the imbalance in the mesolimbic-mesocortical pathway. The probable involvement of several neurotransmitter systems in the pathophysiology of schizophrenia strengthens the assumption of multiple underlying aetiologies in this clinical diagnosis. Moreover, it has been reported that the administration of ad-on SSRIs to a subgroup of patients suffering predominantly from negative symptoms leads to a relief of symptoms (Sepehry, Potvin, Elie, & Stip, 2007).

A potential clinical value of the LDAEP as a biomarker for serotonergic activity would be that subgroups of patients could be identified and treatment decisions could be adapted (Fitzgerald et al., 2009; Linka, Sartory, Gastpar, et al., 2009). So far, only a few studies have assessed the LDAEP in a schizophrenic sample, and most have focused on diagnostic categories. Patients with a diagnosis of schizophrenia (Juckel et al., 2003; Juckel, Gudlowski, et al., 2008; Park et al., 2010) or at-risk for schizophrenia (Gudlowski et al., 2009) showed weaker LDAEP values than healthy subjects, supporting the hypothesis of higher serotonergic levels in schizophrenia. Furthermore, the LDAEP normalized to levels found in healthy subjects after a 4-week treatment with 5-HT_2 antagonists clozapine or olanzapine (Juckel, Gudlowski, et al., 2008). One study by Ostermann et al. (2012) did not find any significant group differences between patients with schizophrenia and healthy subjects regarding LDAEP. Only by considering the symptom dimensions, did patients with schizophrenia spectrum disorders show positive correlations of LDAEP with depression, paranoid ideation, and psychoticism (Ostermann et al., 2012).

Therefore, study I (chapter 5.1) focused on LDAEP and its relationship to the symptom dimension, specifically in patients with chronic schizophrenia suffering mainly negative symptoms.

3. Aims and research questions

The primary aim of this dissertation was to increase our understanding of the problems related to the validation of the LDAEP as a proposed biomarker for central serotonergic activity.

The motivation for this aim is based on previous research focusing on strategies and suggestions to yield clinically relevant biomarkers. Although the LDAEP has been proposed to be a valid marker with predictable power for the serotonin neurotransmitter system, the review of the pertinent research literature identified the following research gaps.

On the one hand, the validation of the LDAEP is compromised when applied to currently used diagnostic categories because they subsume clinical entities of very different aetiologies and pathophysiologies under the same heading. As a result, previous research investigating the LDAEP across patient samples with the same psychiatric diagnosis showed partially contradictory findings. In schizophrenia, both increased and equal serotonergic levels compared to healthy subjects were found (Juckel, Gudlowski, et al., 2008; Ostermann et al., 2012; Park et al., 2010). This could be due to differential biological processes underlying schizophrenia that manifest in positive and negative symptoms and can be explained by a dysfunctional dopaminergic as well as serotonergic system. However, the LDAEP as a biomarker can be used as a powerful tool to represent the biological pathways of brain function and disentangle subsamples of psychiatric disorders more sensitively and specifically than the clinical phenotype. But this problem has to be accounted for by the use of dimensional measures of psychopathology or on measures that record the individual symptoms. **In the first study**, we therefore applied the LDAEP paradigm to a psychiatric sample suffering from schizophrenia, in due consideration of defined subgroups of negative and positive symptoms within this diagnostic group.

On the other hand, the neurobiological mechanisms of the LDAEP are not fully elucidated. It is particularly unclear which neural generators contribute to the EEG waveforms elicited at the scalp during the auditory N1/P2 component. In order to correctly apply the analysis methods such as dipole source analysis or single electrode estimation, the sources underlying loudness perception must be known. Only then can the main claim to what's being measured be satisfactorily met. Moreover, the variety of methodologies used in LDAEP analysis complicates the interpretation and comparability of the results. Thus, **the second study** focused on the investigation of the temporal activation patterns of the generators involved in loudness perception by means of a distributed source model using MEG. The findings of this study may clarify the underlying mechanisms of the LDAEP and potentially improve the accuracy, i.e. validity of the LDAEP.

4. Methods

The following sections give a brief introduction into the methods used in the two studies presented in this thesis investigating the LDAEP, namely electroencephalography (EEG) and magnetoencephalography (MEG). These electrophysiological measures are ideal for examining the function of the auditory cortex, because they do not emit acoustic noise themselves, as would be the case in fMRI for example. EEG and MEG directly reflect the brief, transient changes in neural activity generated through postsynaptic potentials, whereas fMRI measures the hemodynamic response that can only be viewed as an indirect measure of neural activity. Another favourable advantage is its excellent time resolution in the range of milliseconds, providing insight into the temporal dynamics of brain processes (Hansen, Kringelbach, & Salmelin, 2010; Jäncke, 2005).

4.1 Electroencephalography (EEG)

EEG recordings of electrical activity of the human brain are traditionally acquired with electrodes placed on the scalp. These electrodes record the summation of tens of thousands nerve cell potentials in large populations of neurons spreading passively throughout the brain, as well as cerebrospinal fluids, skull and the scalp. The oscillations of brain electric potentials result from chemical processes that trigger charges in the membranes. During the transmission of a signal from one neuron to another, neurotransmitters are released in the synaptic cleft to exhibit or inhibit the target neuron. Glutamate and gamma-aminobutyric acid (GABA) are the main neurotransmitters involved in the generation of excitatory - and inhibitory postsynaptic potentials (EPSP/IPSP), whereas serotonin, dopamine, and acetylcholine have a regulative effect on the reagibility of these neurons (Kandel, Schwartz, & Jessell, 2000). The resulting shift of electrical charges along the cell membranes causes a sink-source configuration in the extracellular medium around the neuron (called passive ohmic current or volume current) that acts as a small dipole, which is derived on the cortical surface. Since the current of a single nerve cell would be too small to be detected at the surface of the scalp, an EEG signal reflects the activation of synchronized assemblies of parallel-organized cortical pyramidal cells (Jäncke, 2005; Michel, 2009).

A powerful tool in neuroscience consists of ERPs, which are derived from on-going EEG activity, averaged over a series of trials, triggered by the same event (such as the presentation of an auditory stimulus) (Walter, Cooper, Aldridge, McCallum, & Winter, 1964). The on-going EEG reflects a wide-range of neural activity that simultaneously regulates sensory and cognitive functions, but also physiological processes. The response of a single sensory stimulus is not visible in the EEG record-

ing, because it is intermixed with noise (background brain activity) and overlaid by stronger physiological signals. By means of averaging several epochs that are time-locked to the stimulus, any random fluctuations will cancel each other out and an increased SNR for the signal of interest can be achieved (Fabiani, Gratton, & Coles, 2000). This is the traditional view to explain the generation of ERPs. An alternative hypothesis claims that ERPs depend on spatial synchronization of underlying neural activity (Makeig et al., 2002; Musall, von Pföstl, Rauch, Logothetis, & Whittingstall, 2014; Sauseng et al., 2007). At rest, the on-going oscillation activity produces signals that are out of phase. During stimuli presentation the phases of this on-going activity are resetted by the stimuli, resulting in a phase-coherent rhythm that can be detected in an ERP (Makeig et al., 2002).

Only the ERPs relevant to this thesis will be discussed, namely the long latency auditory evoked potentials. The most common N1 and P2 components appear approximately 100 ms and 200 ms, respectively, after the presentation of a stimulus (see Fig. 1, panel B). According to a traditional definition they are called exogenous components and are presumed to primarily reflect basic processing, dependent upon characteristics of the external stimulus (e.g. intensity level). However, they are also called transient exogenous components, similar to endogenous components, because internal cognitive processing (e.g. attention) influences their amplitude (Fabiani et al., 2000; Jäncke, 2005).

Although the time resolution capabilities of EEG, in comparison to PET or fMRI, are exceptional, there is a basic limitation regarding the determination of the sources generating neural activity. The exact reconstruction of the location and distribution of neural generators is not conclusive, and this is commonly referred to as the inverse problem (Jäncke, 2005). Theoretically, an infinite number of solutions may result from inferring the generating sources of the known scalp distribution. Only the introduction of constraints on the possible solutions can solve this problem. In order to improve the source estimations, mathematical advances during the last decades have been used to develop some models with appropriate assumptions. These inverse solution algorithms range from dipole source analysis to distributed source models that allow a three-dimensional map of the brain (Michel et al., 2004).

4.2 Source imaging

This section describes some of the currently available source localization approaches in EEG and MEG. Dipole source analysis is most commonly used to measure the LDAEP and was applied in study I included in this thesis (see chapter 5.1). The LORETA approach is also adopted in LDAEP research, albeit less commonly (Jaworska, Blier, Fusee, & Knott, 2012; Jaworska et al., 2013; Mulert, Juckel, Augustin, & Hegerl, 2002; Mulert et al., 2007; Park, Kim, Kim, Im, & Lee, 2011).

Finally, magnetic field tomography (MFT) is a distributed source analysis with minimal priors and most appropriate for exploring the underlying sources in loudness perception. Therfore, the MFT was applied in study II (see chapter 5.2).

4.2.1 Dipole source analysis (DSA)

The main principal of DSA is to specify a small number of sources in a model and test if the resulting scalp potential fits the original measurements. The analysis uses an iterative process to find the best-fitting location of the dipoles that contribute to the scalp potentials that are actually measured, with the lowest residual variance. The projection of neuronal activity from the sources to the surface that reverts to physical assumptions is straightforward (the so-called forward model) (Michel et al., 2004; Scherg, 1990). Because the electric signal travels through the whole brain, the different conductivities of the tissues (brain, CSF, skull and scalp) must be included in the model. Besides some simplified head models that approximate the head with a sphere of homogeneous conductivity or with several spherical shells with different conductivities, more realistic models from individual MRT-scans are used (Jäncke, 2005). The brain electrical source analysis (BESA) software developed by Scherg and Von Cramon (1986) is based on spatiotemporal multiple dipole modelling of discrete sources that can be modelled over a period of time rather than just at one instant. Additionally, this approach considers the orientation of the source (tangential or radial) and allows disentanglement of the overlap inherent in scalp potential waveforms. This is especially advantageous in separating the primary and secondary auditory cortex when analysing the LDAEP (Hegerl & Juckel, 1993). A crucial issue when using dipole models is to assume the correct number of sources. This is mainly based on physiological knowledge. In LDAEP research, for example, two dipoles are set in the auditory cortex of each hemisphere representing the primary and secondary auditory cortex (for an explanation of the detailed procedure refer to Hegerl et al. (1994)). However, a third contributing source is assumed to improve the residual variance of the model (Bruneau, Roux, Garreau, & Lelord, 1985; Näätänen & Picton, 1987) and was implemented in the BESAs tutorial on "AEP experiment on intensity dependence" by Scherg and Hoechstetter (2009). In study I presented in this thesis the expanded dipole model with three dipoles was applied and in study II the physiological basis for these assumptions was explicitly treated.

4.2.2 Low resolution electromagnetic tomography (LORETA)

Although the following source analysis was not applied in the studies presented in this thesis, but was implemented in some studies examining the LDAEP, a short description of its main assumptions is given in the following sections.

LORETA (Pascual-Marqui, Michel, & Lehmann, 1994) is a distributed source model that has the advantage of not being dependent on a priori assumptions about the underlying sources. This means that the solution is minimally biased by the experimenter and each point in a 3D grid is considered as a possible location of a current source. In principle, the inverse solution can be calculated for each voxel in the brain millisecond by millisecond. Unfortunately, according to the inverse problem different constellations of solution points can lead to the same distribution of electrical potentials at the scalp. Indeed, the LORETA approach implements mathematical constraints based on physiological principles that state that the activity of neightbouring neurons is similar. An exact description of the mathematical algorithm can be found in Pascual-Marqui (1999). Studies that investigated the LDAEP by means of the LORETA approach in order to directly obtain the activity in the PAC implemented the probabilistic maps by Penhune, Zattore, MacDonald, & Evans (1996) to define their regions of interests.

4.2.3 Magnetic field tomography (MFT)

Similar to LORETA, the magnetic field tomography (MFT) is based on the minimum norm (MN) algorithm originally proposed by Hämäläinen and Illmoniemi (1994). MFT is able to identify multiple sources at a time, and provides probabilistic estimates for the primary current density within the subject's brain for each time slice independently (Dammers & Ioannides, 2000; Ioannides, Bolton, & Clarke, 1990). Notably, the MFT allows the detection of superficial as well as deeper generators in the brain without fixing the number of generators a priori. The capability of reproducing sources from deeper structures is possible because in contrast to the MN approach, the MFT additionally uses a non-linear weighting function that favours the solution that was initially found. Further, the reconstruction of these initial values improves due to an iterative process. The detailed mathematical algorithm is described in Ioannides et al. (1995). Moreover, MFT results can easily be assigned to anatomical structures when combined with MRI anatomical scans. The resulting 4D spatio-temporal profiles can then be entered in statistical analyses similar to the ones proposed for fMRI. In our case we used Monte Carlo permutation testing, implemented in FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). This test is a subset of non-parametric statistics and assumes no parametric distribution. The standard approach to permutation tests is to exchange the units of the observed data points to obtain the distribution of the test statistic under the null hypothesis. The randomized samples can then be fitted to a general linear model to test the linear combination of explanatory variables (e.g. 4D responses to presented auditory stimuli). In order to assess the neuronal activity between different experimental conditions, i.e. intensity levels, a t-statistic can be calculated. A further advantage of permutation testing is that it allows an exact control of the family-wise error (FWE) rate. This is very important in multiple comparisons because when using a

conventional alpha threshold (typically 5%) in a data set containing thousands of voxels, the probability of false positives will increase enormously (Nichols & Holmes, 2002; Pantazis & Leahy, 2010).

4.3 Comparison of EEG and MEG

In principle, EEG and MEG measurements result from the same type of brain activity. The magnetic field recorded in MEG is evoked by the electrical activity of the postsynaptic intracellular and extracellular currents in pyramidal cells, whereas in EEG mainly extracellular currents contribute to the signal (Hämäläinen, Hari, Ilmoniemi, Knuutila, & Lounasmaa, 1993). Compared to EEG, the spatial resolution of MEG is superior, because the cerebral and extracerebral tissues are transparent to magnetism and thus the attenuation of the signal is merely minimal (Dammers & Ioannides, 2000). Furthermore, no reference point is needed in MEG analysis, whereas in EEG the choice of a reference electrode is essential. In contrast to EEG that is sensitive to tangentially and radially oriented sources, MEG is mostly sensitive to tangentially oriented dipoles (Ahlfors, Han, Belliveau, & Hämäläinen, 2010). In other words, sources that are oriented perpendicular (radial) to the overlying skull (e.g. the top of a gyrus) evoke a magnetic field that is parallel to the sensors and which is not detectable (Fig. 2).

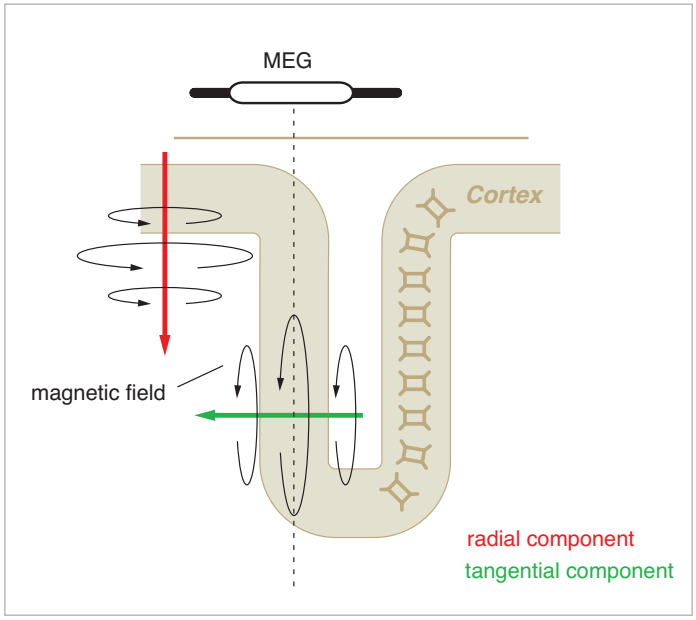

Fig. 2. Schema of a part of the cortex showing a sulcus and two gyri. Sources at the top of a gyrus cause radial components evoking a radial magenetic field that are not detectable by MEG. Adapted from *An introduction to the event-related potential technique* (p. 30), by S. J. Luck, 2005, Cambridge, MA: MIT press. Copyright 2005 by Massachusetts Institute of Technology.

However, when superficial and tangential sources (e.g. within a sulcus) are targeted, the sources can be localized with more accuracy than with EEG, because the signal is contaminated less by disturbing signals. As all primary sensory areas are located on the wall of a sulcus, MEG has been widely used to study processes in the somatosensory and auditory systems (Hämäläinen et al., 1993). Besides these inherent difficulties, the costs of maintenance and the facility to run an MEG are rather high. However, technological advances promise some cost-effective alternatives, for example by replacing the liquid helium, used for cooling the sensors, with liquid nitrogen (Dammers et al., 2014).

5. Empirical part

5.1 Study I: Serotonergic dysfunction in schizophrenia

Title

The loudness dependence of auditory evoked potentials (LDAEP) as an indicator of serotonergic dysfunction in patients with predominant schizophrenic negative symptoms

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5.1.1 Abstract

Besides the influence of dopaminergic neurotransmission on negative symptoms in schizophrenia, there is evidence that alterations of 5-HT system functioning also play a crucial role in the pathophysiology of these disabling symptoms. From post mortem and genetic studies on patients with negative symptoms a 5-HT dysfunction is documented. In addition atypical neuroleptics and some antidepressants improve negative symptoms via serotonergic action. So far no research has been done to directly clarify the association between the serotonergic functioning and the extent of negative symptoms. Therefore, we examined the status of brain 5-HT level in negative symptoms in schizophrenia by means of the loudness dependence of auditory evoked potentials (LDAEP). The LDAEP provides a well established and non-invasive in vivo marker of the central 5-HT activity. We investigated 13 patients with schizophrenia with predominant negative symptoms treated with atypical neuroleptics and 13 healthy age and gender matched controls with a 32-channel EEG. The LDAEP of the N1/P2 component was evaluated by dipole source analysis and single electrode estimation at Cz. Psychopathological parameters, nicotine use and medication were assessed to control for additional influencing factors. Schizophrenic patients showed significantly higher LDAEP in both hemispheres than controls. Furthermore, the LDAEP in the right hemisphere in patients was related to higher scores in scales assessing negative symptoms. A relationship with positive symptoms was not found. These data might suggest a diminished central serotonergic neurotransmission in patients with predominant negative symptoms.

5.1.2 Introduction

Negative symptoms are core features of schizophrenia and are generally considered in psychiatric scales (Andreasen, 1989) and diagnostic classification, e.g. in the DSM-IV-TR (American Psychiatric Association, 2000). These symptoms describe a deficit or an absence of normal mental functions and have traditionally been considered to consist of affective flattening, alogia, avolition, anhedonia and attentional impairment (Andreasen, 1982). Research in this field has characterised negative symptoms to occur as accompanying symptoms of positive symptoms (e.g. hallucinations, delusion and formal thought disturbances) and both in the prodromal and residual state of the disease. They are named primary negative symptoms if directly related to the disease process itself and not resulted in a secondary action from other psychiatric symptoms or medication side effects (Kirkpatrick et al., 2006). Negative symptoms often lead to social impairment, resulting in poor success in social and professional life and account for much of the long-term morbidity and poor functional outcome of patients with schizophrenia (Häfner, Löffler, Maurer, Hambrecht, & an der

Heiden, 1999; Rössler et al., 2005). Despite that, the pathophysiology of negative symptoms has been widely unknown so far.

Schizophrenia research on biochemical functions has largely focused on the dopamine neurotransmitter system. The dopamine-hypothesis based on imaging studies proposes an imbalance of cortical and subcortical preponderance of dopaminergic neurotransmission, where a subcortical hyperstimulation of dopamine D_2-receptors leads to positive symptoms, and a hypoactivation of cortical dopamine D_1-receptors leads to negative and cognitive symptoms (K. L. Davis et al., 1991; Grace, 1991; Knable & Weinberger, 1997; Weinberger, 1987). However, the theory of a serotonin (5-HT) and dopamine interaction as the mechanism behind schizophrenia has gained more acceptance. Moreover, there is evidence that the serotonin system inhibits dopamine function in frontal cortex and reinforces the imbalance in the mesolimbic-mesocortical pathway of the dopaminergic system (Abi-Dargham, 2007; Breier, 1995; Kapur & Remington, 1996; Meltzer, 1989; B. Roth & Meltzer, 2000). The involvement of the serotonergic system in this theory is due to the fact that atypical neuroleptics (J. M. Davis, Chen, & Glick, 2003; Leucht, Heres, Kissling, & Davis, 2011; Miyamoto, Duncan, Marx, & Lieberman, 2004) and antidepressants (Rummel-Kluge, Kissling, & Leucht, 2006; Silver, 2004; S. P. Singh, Singh, Kar, & Chan, 2010), which act via the serotonergic system, show remarkable potency for the treatment of negative symptoms. Meltzer (1999) specifies that 5-HT_{2A} receptor antagonism and 5-HT_{1A} partial agonism together with weak dopamine D_2 receptor antagonism are responsible for the principal pharmacologic effects of atypical neuroleptics on negative symptoms. An addendum to the former concept was that this new hypothesis allows explaining the heterogeneity of schizophrenia even better. Since a single type of abnormality of the neurotransmitter systems is unlikely to emerge as characteristic of all patients with schizophrenia. To sum up, there is evidence that the serotonergic system is a key component in the pathogenesis of negative symptoms.

The serotonin system plays an important role in pathophysiology of the major psychiatric disorders and provides a target of pharmacotherapeutic interventions. Therefore reliable indicators of this system are in urgent need for clinical and scientific interest (Hegerl & Juckel, 2000). Such indicators could be used after overcoming some challenges concerning the implementation in daily clinical use to identify patients with serotonergic dysfunctions and thus serve as therapy predictors (Hegerl et al., 2001; Juckel, Pogarell, et al., 2007). In fact, common indicators of the serotonin system are mainly indirect peripheral parameters that only give an approximate indication of the central serotonergic system. Such methods as neuroendocrinological challenge tests, measuring concentrations of serotonin metabolites in cerebrospinal fluid and tryptophan depletion test have not been proven to be sufficiently valid (Juckel, 2005). Furthermore, the use of imaging techniques that allow to reflect the availability of binding potentials of serotonin transporter (SERT) or 5-HT receptors, such as positron emission tomography (PET) (Quednow et al., 2012) and single photon emission computed tomogra-

phy (SPECT), are not appropriate for daily clinical use because of their invasive properties (B. Roth & Meltzer, 2000).

In the continuing search for biological correlates of psychiatric disorders, evoked potentials now constitute a prime target of investigation. In particular, the loudness dependence of auditory evoked potentials (LDAEP) has been widely reported to be a valid measure of central serotonergic activity in humans (Hegerl et al., 2001; Hegerl & Juckel, 1993, 1994; Kawohl, Hegerl, Müller-Oerlinghausen, & Juckel, 2008; I. H. Lee et al., 2011; O'Neill, Croft, et al., 2008). This measure represents a growth of the amplitude in primary auditory cortices, measured from the peak of the N1 to the peak of the P2 component along with an increase in sound pressure level (Fig. 3A). A pronounced LDAEP supposedly reflects a low central serotonergic neurotransmission and vice versa. Some other reports have suggested that the interpretation may be more complex and the LDAEP's specificity as a marker of serotonin function has been challenged (Hitz et al., 2011; Juckel, Kawohl, et al., 2008; Kawohl, Giegling, et al., 2008; O'Neill et al., 2007).

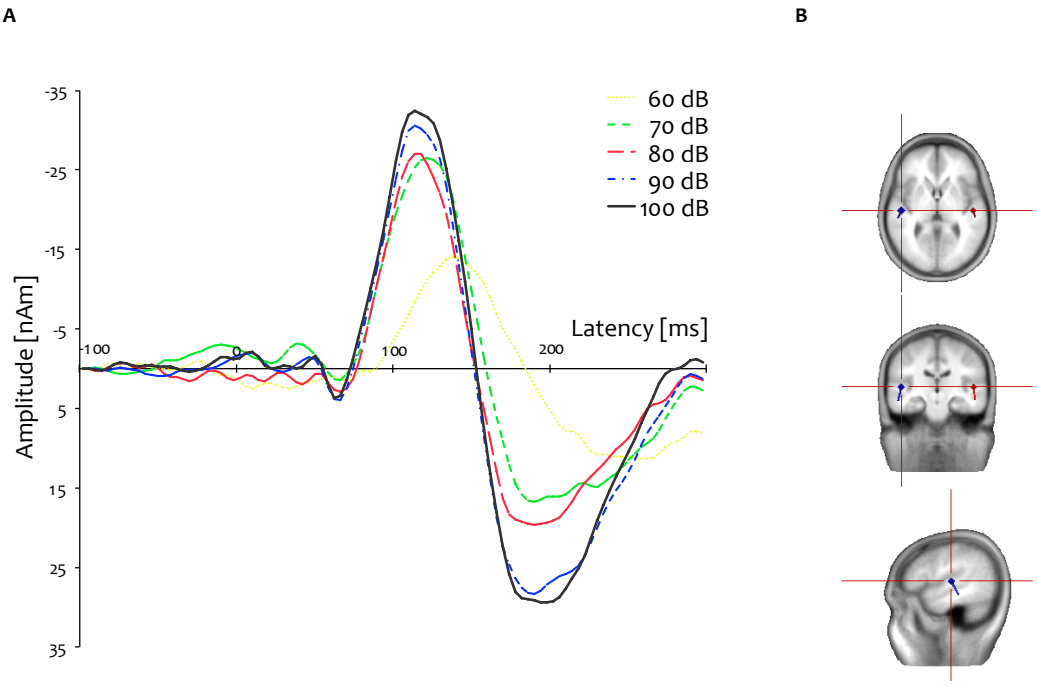

Fig. 3. A) Example of loudness dependence of auditory evoked potential (LDAEP). Auditory evoked activity of the tangential dipole in the right hemisphere following auditory stimulation of a 1000 Hz tone with different sound pressure levels (60 to 100 dB SPL) over all subjects (n=26). B) Dipole sources of auditory evoked potentials localized in primary auditory cortex.

A significant body of research documents a weaker LDAEP in patients with schizophrenia compared to healthy controls, thus indicating increased serotonergic activity in patients (Gudlowski et al.,

2009; Juckel et al., 2003; Juckel, Gudlowski, et al., 2008; Park et al., 2010). But this research to date has tended to focus on the diagnosis of schizophrenia, neglecting the clinical heterogeneity. However, it is of great interest to investigate schizophrenia on the psychopathological symptom level. Thus, the aim of the current study is to scrutinize the putative role of serotonergic neurotransmission of negative symptoms in schizophrenia.

5.1.3 Methods

5.1.3.1 Subjects

The sample included 26 male subjects (13 patients, 13 controls) who underwent electrophysiological recording. Subjects with psychiatric comorbidity, drug or alcohol abuse, benzodiazepine consumption for more than 10 days before examination or a lifetime history of neurological diseases were excluded. Thirteen patients with predominant negative symptoms recruited from the Department of General and Social Psychiatry at the Psychiatric University Hospital Zurich met the diagnostic criteria for chronic paranoid schizophrenia in accordance to ICD-10 (World Health Organization, 1993). The psychopathological state of all patients was rated based on the Positive and Negative Syndrome Scale (PANSS; Kay, Fiszbein, & Opler, 1987) and the Scale for Assessment of Negative Symptoms (SANS; Andreasen, 1989). To differentiate depressive symptoms from negative symptoms, Hamilton Depression Rating Scale (HAMD; Hamilton, 1960), Bech-Rafaelsen Melancholia Scale (BRMS; Bech & Rafaelsen, 1980) and Calgary Depression Rating Scale for Schizophrenia (CDSS-G; Addington, Addington, & Schissel, 1990) were applied. All patients were using atypical antipsychotics during the test period. Dosages were transformed into chlorpromazine (CPZ) equivalent values for comparative reasons (Möller, Müller, & Bandelow, 2001). Thirteen healthy, age- and gender-matched volunteers recruited from medical staff and students served as the control group. Controls with a lifetime history of any psychiatric disorder were excluded.

5.1.3.2 Ethics statement

The study was approved by the special subcommission for psychiatry of the ethics committee of the canton of Zurich (“SPUK ZH Psychiatrie”) under the title "Die Rolle der zentralen serotonergen Aktivität bei Negativsymptomen" (Ref.-Nr.: E-19/2006) and was carried out in accordance with the Declaration of Helsinki. All subjects have given written informed consent. Only participants with uncompromised capacity to consent were approached. The capacity to give consent had been established by the senior consultant psychiatrists responsible for the treatment of the patients.

5.1.3.3 Electrophysiological assessment

Subjects were seated with their eyes open in a quiet room adjacent to the recording apparatus and were asked to avoid facial muscle movements throughout the auditory stimulus presentation sequence and the recording. As attention to the auditory stimuli has been shown to modulate the auditory evoked potentials (Schechter & Buchsbaum, 1973) and therefore also the LDAEP (Baribeau & Laurent, 1987), a silent movie was shown to them for distraction and the stimuli were presented in randomized orders and points in time that precluded preparatory state. Auditory evoked potential (AEP)-recording was performed with 32 electrodes referenced to FCz (BrainCap-MR 32 standard, 32 channels, Easycap, Herrsching-Breitbrunn) in accordance with the international 10-20 System (Jasper, 1958). Scalp electrode impedances were kept below 10 kΩ. Sinus tones (1 000 Hz, 40 ms duration with 10 ms rise and fall time, ISI randomized between 1 800 and 2 240 ms) of five intensities (60, 70, 80, 90, 100 dB sound pressure level, generated by a PC-stimulator) were presented binaurally in a pseudo-randomized order over headphones using Presentation software (Neurobehavioral Systems, Inc. San Pablo, CA). Data were collected with a sampling rate of 250 Hz and a band pass filter (0.5-70 Hz). Continuous EEG files for each subject were loaded into Brain Electrical Source Analysis software (BESA, version 5.3, MEGIS, Gräfelfing, Germany) and filtered digitally with a high bandpassfilter of 0.16-30 Hz (6/12 dB octave). Before averaging, the first responses of each of the five intensities were excluded in order to reduce short-term habituation effects. For artefact suppression, all trials were automatically excluded from averaging when the voltage exceeded ±50 μV in any of the 32 channels at any point during the averaging period. Data with a 100 ms pre stimulus and a 300 ms post stimulus baseline interval were then inspected visually. On average 63 % (± 5.228) artefact-free sweeps per intensity were averaged separately for each participant, which should lead to an appropriate signal-to-noise ratio.

5.1.3.4 Dipole source analysis (DSA) and single electrode estimation

Dipole source localization of the N1/P2-component of AEPs was computed by means of the inverse solution as implemented in BESA, using a spherical head model. DSA provides an important methodological advance, because overlapping subcomponents of the N1/P2-component in the primary as well as secondary auditory cortex can be studied separately (Scherg, Vajsar, & Picton, 1989). This is a pivotal point, as primary auditory cortex is highly innervated by serotonin compared to secondary auditory cortex (Hegerl et al., 1994). Similar studies reveal a high spatio-temporal accuracy with DSA (Kawohl et al., 2007; Waberski, Gobbele, Kawohl, Cordes, & Buchner, 2003). Based on the grand average over all subjects a dipole model was computed for the 60 dB and 70 dB intensities with two regional sources (one for each hemisphere, Fig. 3B). Several authors suppose a

frontal protective mechanism being activated during presentation of high tone intensities (Bruneau et al., 1985; Yamaguchi & Knight, 1990). Therefore a third regional source was added to the frontal region for the high intensity dipole model computed for the 90 and 100 dB intensities. These two models were applied to the individual data sets (low intensity model to 60-70 dB, high intensity model to 80-100 dB) in order to obtain the spatio-temporal information of the brain activation. The methods have been published in detail elsewhere (Hegerl et al., 1994; Hegerl & Juckel, 1993, 1994).

Because the majority of studies on the LDAEP focused on the N1/P2 component, which seems to be more internally consistent and test-retest reliable than slopes based on other components (Beauducel et al., 2000; Hegerl, Prochno, Ulrich, & Müller-Oerlinghausen, 1988), the peak-to-peak N1/P2 amplitudes were used to quantify differences in the responses to the different tone intensities. Additionally to the DSA approach we analysed the data with a scalp method, as recommended by our group (Hagenmuller, Hitz, Darvas, & Kawohl, 2011), to facilitate across-study comparisons. N1/P2 amplitudes were determined at the Cz electrode and were re-referenced to linked mastoids. The LDAEP was determined by the median of all slopes of each possible connection between the five different N1/P2 amplitudes corresponding to the five different intensities (Hegerl & Juckel, 1994) for tangential dipole activity of both hemispheres and Cz-electrode estimation derived amplitudes. These values were used as the main variables for statistical evaluation.

5.1.3.5 Statistical analysis

Comparison of age and smoking status in patients and controls was conducted with a t-test for independent samples and cross-tabulation with χ^2 test, respectively. To test the association between LDAEP values and the group factor (control group vs. schizophrenic patients) we conducted a series of generalized linear models (GLM) (Hoffman, 2004). GLM was chosen because it allows for variables that are not normally distributed in comparison to familiar used methods as ANOVA or linear regression analysis. LDAEP of the left and right hemisphere and from Cz-estimation were entered as the dependent variables. The covariates age and nicotine use were tested separately in bivariate analyses against LDAEP using DSA. Distribution and link-function of the LDAEP variables were chosen according to their graph and the goodness of model fit indices. For this purpose we compared the Akaike's information criterion (AIC) and the Bayesian information criterion (BIC) for the different distributions and link-functions. The best fit to the data was finally obtained with a gamma distribution (right skewed distribution) and log link-function. In all GLM a robust estimator was used to reduce the effects of outliers and influential observations. Group effects on LDAEP were displayed with mean differences, whereas associations between continuous measures and LDAEP were depicted with unstandardized regression coefficients (B). In order to provide comparability

among predictors all continuous covariates were standardized using the z-transformation. Wilcoxon-test was used to test if the medians between left and right LDAEP differed significantly. Analyses were carried out with SPSS version 20 for Windows.

5.1.4 Results

Demographics and psychopathology data for both groups are summarised in Table 1. Although antipsychotic medication estimated by CPZ-equivalent dose had a medium to strong effect on psychopathological scales, the correlations did not reach the level of statistical significance (PANSS general score; r = -0.698, P = .08; other scales r = -0.31 - 0.44, P > .10).

Table 1. Demographic and clinical data of the sample.

	Patients	**Controls**	**t / χ2**	**P**
N	**13**	**13**		
Age (years)	35.0 (8.13)	35.4 (8.17)	t =0.120, df=24	.905
Medication (CPZ)	707.0 (597.62)	-	-	-
Smoking (yes/no)	69; 31	23; 77	χ2=5.571, df=1	.018 *
PANSS positive	15.46 (4.93)	-	-	-
PANSS negative	18.39 (6.25)	-	-	-
SANS composite score	31.31 (15.39)	-	-	-
BRMS	6.31 (3.77)	-	-	-
HAMD 17	8.69 (4.07)	-	-	-
CDSS-G	3.15 (3.11)	-	-	-

Data presented as % or mean ± SD. Abbreviations: CPZ, Chlorpromazine Dose Equivalence Ratios; PANSS, Positive and Negative Syndrome Scale; SANS, Scale for Assessment of Negative Symptoms; BRMS, Bech-Rafaelsen Melancholia Scale; HAMD, Hamilton Depression Rating Scale, CDSS-G, Calgary Depression Rating Scale for Schizophrenia. * P < .05.

The LDAEP using DSA was significantly associated with the group membership in both hemispheres (right: Wald = 10.094, df = 1, P = .001; left: Wald = 7.791, df = 1, P = .005). Patients with schizophrenia showed a significantly higher LDAEP than the control group (Table 2, Fig. 4). Results were adjusted for age and nicotine use. The magnitude of the group effect on LDAEP on both hemispheres was remarkably large, as indicated through the standardized mean difference Cohen's d

= 1.04 (left) and d = 1.20 (right) (benchmarks are as follows: d = 0.3 depicts a small effect, d = 0.5 a medium effect and d = 0.8 a large effect). No significant differences in the LDAEP between the groups were found using single electrode estimation at Cz (Wald = 0.057, df = 1, P = .811). No significant differences between left and right LDAEP were found, neither among the whole sample (Z = -1.283, P = .200), nor among schizophrenic patients (Z = -1.153, P = .249) or the control group (Z = -.524, P = .600).

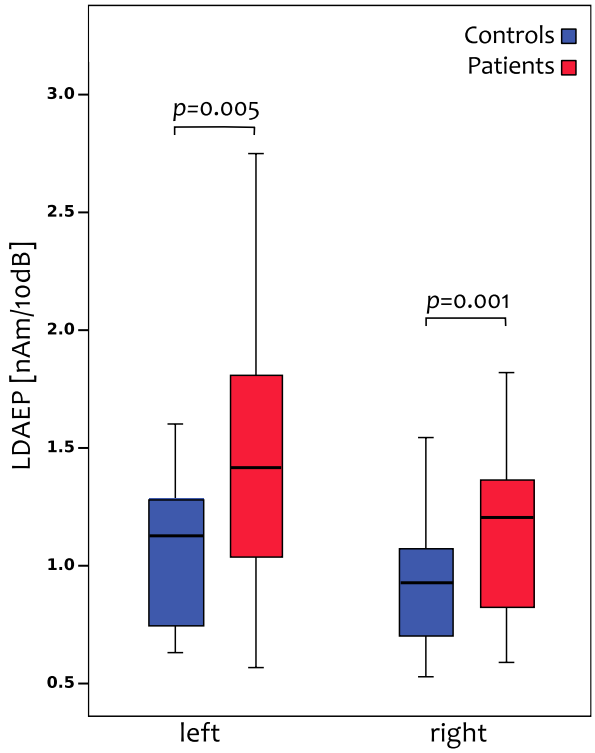

Fig. 4. Overall distribution of the loudness dependence of auditory evoked potentials (LDAEP) values between healthy controls and patients with schizophrenia. The boxplots represent medians, quartiles and extreme values of the LDAEP variable in the left and right hemisphere across both groups.

Table 2. LDAEP mean values in left and right hemisphere and Cz electrode across groups.

Hemisphere	Group	Mean	95% CI	Wald χ^2 (df)	Sig
Left	Controls	1.060	0.894-1.258	7.791 (1)	.005 *
	Patients	1.450	1.230-1.710		
Right	Controls	0.905	0.781-1.050	10.094 (1)	.001 *
	Patients	1.234	1.073-1.420		
Cz	Controls	0.150	0.116-0.194	0.057 (1)	.811
	Patients	0.142	0.105-0.192		

Results are adjusted for age and nicotine use. * p < .01.

Moreover, we observed a significant positive relationship between the SANS subscales "affective flattening" (beta = 0.207, P = .000), "anhedonia" (beta = 0.155, P = .016) and "attentional impairment" (beta = 0.189, P = .015) and the LDAEP in the right hemisphere in patients. SANS composite score (the sum of scores for all items), which reflects severity of negative symptoms, was also positively correlated with the right LDAEP (beta = 0.153, P = .035) (Table 3, Fig. 5A). In figure 5 the association between LDAEP and the severity of negative as well as positive symptoms are illustrated. Depressive symptoms (BRMS and CDSS G scale) (beta = -0.372, P = .000; beta = -0.305, P = .000) as well as PANSS general score (beta = -0.159, P = .026) were associated with the left LDAEP. Patients with higher scores on these scales showed lower LDAEP (Table 4). As shown in Table 3 and 4 all other psychopathological scales were not significant. LDAEP on both hemispheres were positively associated with medication by means of CPZ-equivalent dose (beta = 0.162, P = .001 and beta = 0.173, P = .030 for right and left hemisphere).

Table 3. Associations between right-hemispheric LDAEP values and clinical characteristics among patients.

Measures	**B**	**95%-CI**	**Wald χ^2 (df)**	**Sig**
CPZ	0.162	0.069; 0.254	11.593 (1)	.001 *
PANSS positive	−0.014	−0.154; 0.125	0.041 (1)	.840
PANSS negative	0.103	−0.036; 0.243	2.114 (1)	.146
PANSS composite score	−0.086	−0.241; 0.070	1.170 (1)	.279
PANSS general	−0.174	−0.354; 0.006	3.574 (1)	.059
SANS Affect	0.207	0.094; 0.321	12.908 (1)	.000 *
SANS Alogia	−0.015	−0.151; 0.121	0.047 (1)	.829
SANS Avolition	−0.101	−0.228; 0.026	2.416 (1)	.120
SANS Anhedonia	0.155	0.029; 0.282	5.779 (1)	.016 *
SANS Attention	0.189	0.036; 0.341	5.906 (1)	.015 *
SANS composite score	0.153	0.011; 0.296	4.451 (1)	.035 *
BRMS	0.054	−0.129; 0.237	0.331 (1)	.565
HAMD 17	−0.049	−0.204; 0.106	0.391 (1)	.532
CDSS G	−0.055	−0.184; 0.074	0.695 (1)	.404

Abbreviations: CPZ, Chlorpromazine Dose Equivalence Ratios; PANSS, Positive and Negative Syndrome Scale; SANS, Scale for Assessment of Negative Symptoms; BRMS, Bech-Rafaelsen Melancholia Scale; HAMD, Hamilton Depression Rating Scale, CDSS-G, Calgary Depression Rating Scale for Schizophrenia. B, unstandardized regression coefficient. * P < .05.

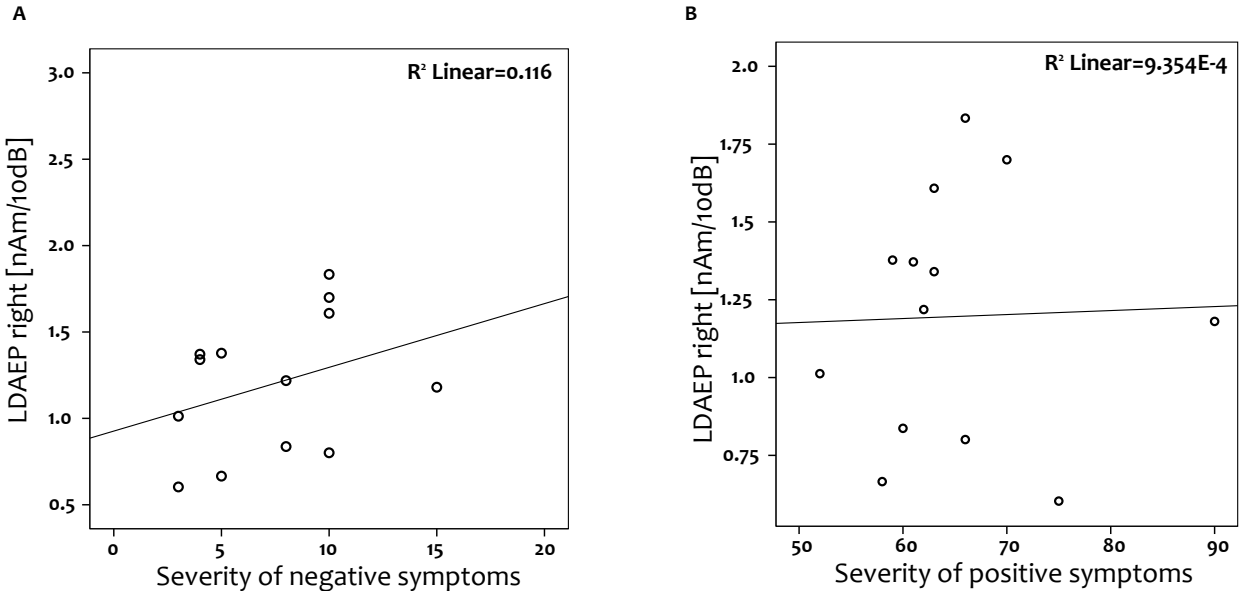

Fig. 5. A) LDAEP of the right hemisphere correlates with severity of negative symptoms in schizophrenic patients ($p < .05$). B) No correlation with positive symptoms was found.

Table 4. Associations between left-hemispheric LDAEP values and clinical characteristics among patients.

Measures	**B**	**95%-CI**	**Wald χ^2 (df)**	**Sig**
CPZ	0.173	0.160; 0.331	4.681 (1)	.030 *
PANSS positive	−0.091	−0.219; 0.037	1.935 (1)	.164
PANSS negative	−0.061	−0.275; 0.152	0.320 (1)	.572
PANSS composite score	−0.007	−0.237; 0.223	0.004 (1)	.950
PANSS general	−0.159	−0.299; −0.019	4.962 (1)	.026 *
SANS Affect	−0.073	−0.256; 0.110	0.607 (1)	.436
SANS Alogia	−0.048	−0.319; 0.224	0.118 (1)	.731
SANS Avolition	−0.120	−0.329; 0.089	1.263 (1)	.261
SANS Anhedonia	−0.219	−0.367; −0.071	8.406 (1)	.004 *
SANS Attention	−0.028	−0.276; 0.221	0.047 (1)	.828
SANS composite score	−0.137	−0.294; 0.019	2.970 (1)	.085
BRMS	−0.372	−0.493; −0.250	36.082 (1)	.000 *
HAMD 17	−0.075	−0.294; 0.143	0.457 (1)	.499
CDSS G	−0.305	−0.409; −0.202	33.331 (1)	.000 *

Abbreviations: CPZ, Chlorpromazine Dose Equivalence Ratios; PANSS, Positive and Negative Syndrome Scale; SANS, Scale for Assessment of Negative Symptoms; BRMS, Bech-Rafaelsen Melancholia Scale; HAMD, Hamilton Depression Rating Scale, CDSS-G, Calgary Depression Rating Scale for Schizophrenia. B, unstandardized regression coefficient. * $P < .05$.

5.1.5 Discussion

The present study was designed to investigate the role of serotonergic neurotransmission estimated by the LDAEP for the psychopathology of negative symptoms in schizophrenia. Due to the heterogeneity of the clinical concept of schizophrenia and its limitations as a valid object for scientific investigation (Cook, 2008), the level of psychopathological symptoms was chosen. We hypothesized that the LDAEP in patients with predominant negative symptoms would deviate from that of patients with predominant positive symptoms and healthy controls, indicating a difference in serotonergic neurotransmission. The results of this study provide new evidence in schizophrenia research. We would like to emphasize two remarkable findings. First, patients with schizophrenia showed a significantly stronger LDAEP than the control group. Based on the presumptions of the inverse relationship between LDAEP and 5-HT, this may indicate a difference in serotonergic neurotransmission. Moreover, the stronger LDAEP in patients with schizophrenia is highly associated with negative symptoms. Second, only the increased LDAEP in the right hemisphere was associated with negative symptoms, underscoring the effects of laterality in brain functions and brain activity in schizophrenia. The single electrode estimation at Cz did not show any significant differences between the groups, which may derive from additional frontal source activation involved in high intensities. This has also been reported by Hagenmuller et al. (2011).

Our findings contrast with those of previous studies, which showed that patients with schizophrenia had a weaker LDAEP than healthy controls (Gudlowski et al., 2009; Juckel et al., 2003; Juckel, Gudlowski, et al., 2008; Park et al., 2010). However, those studies were not designed to control for LDAEP differences between positive and negative symptoms. They focused on schizophrenic patients as a self-contained group. Nevertheless, Juckel, Gudlowski, et al. (2008) reported a tendency toward a positive relationship between PANSS negative score and LDAEP whereas Gudlowski et al. (2009) found a negative relationship between those scores. Our findings are contrary to the results of Gudlowski et al. (2009). One explanation for inconsistent findings could be due to a difference in methodology as sampling biases, gender effects, intensity of stimuli and methods of estimation (DSA vs. single-electrode) (O'Neill, Croft, et al., 2008). In particular, our data were analysed with DSA method, whereas Gudlowski et al. (2009) used single-electrode estimation for LDAEP. According to Hagenmuller et al. (2011), studies using different methods are difficult if not impossible to compare. Furthermore, the sample in Gudlowski's study included females and males. Even though some studies reported no gender effects (Hegerl & Juckel, 1994; Park et al., 2010; Simmons et al., 2011), others have documented some effects on the LDAEP (Hensch et al., 2008; Jaworska et al., 2012; Oliva et al., 2011). The study by Juckel, Gudlowski, et al. (2008) used comparable methodology to the present study and some results are in line with our findings, showing strong LDAEP in patients

with negative symptoms among schizophrenic patients. Compared to healthy controls, they reported weaker LDAEP in the left hemisphere in patients, which states a contrary result to our findings.

Nonetheless, our results are consistent with those of previous research on neurotransmitter alterations in negative symptoms and suggest the role of an impaired serotonergic system (Breier, 1995). Although many studies on the direct involvement of the serotonin system in schizophrenia exist, here we focus on results concerning negative symptoms. Direct evidence is provided from a post-mortem study, which reported a decreased 5-HT_2 receptor density in frontal cortex in patients with chronic schizophrenia (Hashimoto et al., 1993). Furthermore, a PET study showed lower availability of 5-HT_{1A} receptors in patients with schizophrenia compared to healthy controls and receptor binding was negatively associated with negative symptoms, estimated by the PANSS scale (Yasuno et al., 2004). Moreover, a genetic study by Reynolds, Yao, Zhang, Sun, & Zhang (2005) gives support to an involvement of the serotonergic system in the pathogenesis of negative symptoms, since the 5-HT_{2C} receptor promoter polymorphism is associated with negative symptoms. From studies on the mechanism of action of atypical neuroleptics in negative symptoms an indirect evidence for serotonergic involvement is provided. In this context, it remains unclear why serotonin antagonists as well as agonists have an impact on the serotonergic system and improve the outcome of negative symptoms. Silver (2004) suggests that these pharmacologically distinct treatments may share common final mechanism. This paradoxical finding needs further investigation. Moreover, one has to consider that different 5-HT receptors have opposite effects on the function of neurons by means of inhibition and excitation (B. Roth & Meltzer, 2000). Further research is needed to clarify, if negative symptoms are caused directly by a primary abnormality in serotonergic transmission or in a secondary way via modulation of dopamine release (Abi-Dargham, 2007; Goff & Evins, 1998; Winograd-Gurvich, Fitzgerald, Georgiou-Karistianis, Bradshaw, & White, 2006).

With regard to the laterality effect, the present results showed a positive association between LDAEP and negative symptoms (SANS subscale affective flattening, anhedonia and attentional impairment, SANS composite score) in the right hemisphere, and a negative association between LDAEP and depressive symptoms (BRMS and CDSS G scale) in the left hemisphere in schizophrenic patients. A possible explanation could be that the LDAEPs in patients with high scores on depressive scales converge towards the LDAEP values of healthy controls (weaker LDAEP). This conclusion is in line with the literature about LDAEP in depressive patients, where no significant effect on the LDAEP has been shown (Jaworska et al., 2012; Linka, Müller, Bender, & Sartory, 2004; Park et al., 2010). Our results could also be due to serotonergic interhemispheric asymmetry, respectively to a reduced leftward asymmetry of brain structures of the auditory cortex in schizophrenia as observed by Salisbury, Kuroki, Kasai, Shenton, & McCarley (2007) and Shenton, Dickey, Frumin, & McCarley (2001). At that juncture, that the role of the laterality effect in schizophrenia and in particular in

negative symptoms is not known, interpretation is limited.

Nevertheless, our study is not without limitations: The sample size was relatively small, which had an effect on the statistical power. Furthermore, the effects of education were not considered. With regard to the influence of attention to the LDAEP (Baribeau & Laurent, 1987) and given that patients with negative symptoms often show an attention deficit during auditory performances (Bozikas, Kosmidis, Kioperlidou, & Karavatos, 2004), an objective procedure controlling attention would have been necessary to add further consistency to our findings. A biased effect of medication is plausible since all patients were treated with atypical neuroleptics. There was an association with CPZ-equivalent dose and LDAEP found in both hemispheres in this study, indicating an elevated LDAEP (and lower serotonergic activity) with higher medication use. Furthermore, general symptoms rated on PANSS scale were negatively related to medication in that they displayed a statistical trend ($p=0.08$). In a study by Juckel et al. (2003) an increased LDAEP after a treatment with atypical neuroleptics compared to baseline was observed. Moreover, in a PET study, a trend toward a decreased 5-HT_2 receptor binding in prefrontal cortex was found in neuroleptic treated patients, whereas neuroleptic naive patients showed similar results as healthy controls (Okubo et al., 2000). As negative symptoms also occur as pharmacological side effects (secondary negative symptoms) it is debatable if the found relationship between LDAEP and negative symptoms is an effect of secondary negative symptoms. A distinction between primary and secondary negative symptoms is not possible with contemporary measurements of psychopathology (Flaum & Andreasen, 1995; Lindenmayer et al., 2007). On the other hand, a study design including unmedicated chronic schizophrenic patients is hardly realistic both for ethical reasons and practicability. Further studies with more focus on the effect of medication are therefore needed.

Another limitation is the possible influence of other neurotransmitters on the LDAEP. There are genetic association studies and challenge trials on possible influences of dopamine, glycine, and nitric oxide (Hitz et al., 2011; Juckel, Kawohl, et al., 2008; Kawohl, Giegling, et al., 2008; O'Neill et al., 2007). As these studies point to a sensitivity of the LDAEP also to neurotransmitter systems other than 5-HT, the LDAEP's specificity as a marker of serotonergic function is challenged (O'Neill, Croft, et al., 2008). This has to be taken into account in the interpretation of this study. Nevertheless, also these results are in part heterogeneous, e.g. an association of the LDAEP with the dopaminergic system by means of the COMT Val158Met-polymorphism (Juckel, Kawohl, et al., 2008) could not be reflected in a dopaminergic challenge trial (Hitz et al., 2011).

In conclusion, the aim of the present study was to investigate the LDAEP as an indicator of serotonin functioning within the schizophrenic spectrum. In particular, we took account of the heterogeneity of clinical diagnosis by examining the accurate psychopathological symptoms. The results showed an

association between the serotonergic function estimated by the LDAEP and the extent of negative symptoms directly. Our findings support the idea of differential clinical features of schizophrenia and contribute to the clarification of the aetiology of negative symptoms.

5.2 Study II: Underlying mechanisms of the LDAEP

Title

Spatiotemporal properties of auditory intensity processing in multisensor MEG

Authors

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5.2.1 Abstract

Loudness dependence of auditory evoked potentials (LDAEP) evaluates loudness processing in the human auditory system and is often altered in patients with psychiatric disorders. Previous research has suggested that this measure may be used as an indicator of the central serotonergic system through the highly serotonergic innervation of the auditory cortex. However, differences among the commonly used analysis approaches (such as source analysis and single electrode estimation) may lead to different results. Putatively due to discrepancies of the underlying structures being measured. Therefore, it is important to learn more about how and where in the brain loudness variation is processed. We conducted a detailed investigation of the LDAEP generators and their temporal dynamics by means of multichannel magnetoencephalography (MEG). Evoked responses to brief tones of six different intensities were recorded from 19 healthy participants. We used magnetic field tomography in order to appropriately localize superficial as well as deep source generators of which we conducted a time series analysis. The results showed that apart from the auditory cortex other cortical sources exhibited activation during the N1/P2 time window. Analysis of time courses in the regions of interest revealed a sequential cortical activation from primary sensory areas, particularly the auditory and somatosensory cortex to posterior cingulate cortex (PCC) and to premotor cortex (PMC). The additional activation within the PCC and PMC has implications on the analysis approaches used in LDAEP research.

5.2.2 Introduction

The loudness dependence of the N1/P2 auditory evoked potentials (LDAEP) constitutes a prime object of investigation in the continuing search for biological correlates of psychiatric disorders (Kenemans & Kähkönen, 2011). Due to feasibility reasons the LDAEP is mostly recorded by electroencephalography (EEG). Nonetheless, the loudness dependence can also be measured from changes in magnetic fields (LDAEF) using magnetoencephalography (MEG). LDAEP has been reported to be a valid measure of central serotonergic neurotransmission both in animals and humans (Hegerl & Juckel, 1993; O'Neill, Croft, et al., 2008). The basic concept of LDAEP is that serotonergic neurotransmission has a homeostatic function and modulates the responsiveness and sensitivity of cortical neurons in the primary auditory cortex (PAC) (Jacobs & Azmitia, 1992). A pronounced LDAEP supposedly reflects low central serotonergic neurotransmission, whereas a weak LDAEP reflects high serotonergic activity (Hegerl & Juckel, 1993; Kawohl, Hegerl, et al., 2008; Wutzler et al., 2008). The measure of the auditory evoked response to increasing sound pressure levels is referred to as loudness dependence and is inter-individually different (Buchsbaum, 1971). The LDAEP has been widely applied in psychiatric samples (Ostermann et al., 2012; Park et al.,

2010; Wyss et al., 2013), in studies with pharmaceutical challenge (Hitz et al., 2011; Kähkönen, Jääskeläinen, Pennanen, Liesivuori, & Ahveninen, 2002), in relation with the prediction of treatment outcome (Juckel, Pogarell, et al., 2007; Linka et al., 2004) and in genetic association studies (Gallinat et al., 2003; Juckel, Kawohl, et al., 2008; Kawohl, Giegling, et al., 2008).

Nonetheless, further improvement of the sensitivity and specificity of the LDAEP analysis is required to qualify it as a diagnostic marker. This could facilitate the clinical applicability of the parameter. Thus far the LDAEP has been analyzed in several different ways. The most common strategies used in EEG rely on single-electrode estimation (e.g. Debener et al., 2002), dipole source analysis (DSA; e.g. Wyss et al., 2013) and current source density analysis using low resolution brain electromagnetic tomography (LORETA; e.g. Jaworska et al., 2013). A general restriction of source localisation in EEG and MEG is the existence of the inverse problem (Michel et al., 2004). This indicates that from the signal measured at the scalp, the location of the underlying generators is not uniquely determined. One should keep in mind that different source configurations can produce the same potentials on the scalp.

By using the EEG single-electrode estimation at the Cz electrode or the Fz electrode – by far the most used strategy to define the LDAEP – we have to account for following limitations: first, it is for sure that a single electrode cannot adequately represent source activity generated by the underlying brain area due to the superposition effect and the poor conductance of the skull. Secondly, using a single channel only is not possible to set apart the overlapping generators in both the primary and secondary auditory cortices with this approach. This differentiation is important because the serotonin concentration is highest in the primary sensory areas (Azmitia & Gannon, 1986; Juckel et al., 1997; Lewis, Campbell, Foote, & Morrison, 1986). Positron emission tomography (PET) and autoradiography analysis showed a high mean density of serotonin transmitter receptors in the PAC (Fink et al., 2009; Zilles et al., 2002). Moreover, a functional magnetic resonance imaging (fMRI) study on LDAEP using individual landmarks for the separation of primary and secondary auditory cortices indicated different loudness dependencies in these areas (Brechmann, Baumgart, & Scheich, 2002). Thus, the primary auditory cortex plays a pivotal role in the analysis of the LDAEP and the consequential interpretations.

Fortunately, advances in source reconstruction allow for the isolation of the signal from the PAC in a relatively straightforward fashion. Both dipole source analysis (Hegerl & Juckel, 1993; Scherg & Picton, 1991) and LORETA (Mulert et al., 2002) showed to be capable of separating the sources in the auditory cortex and are used to determine the LDAEP. One problem with DSA is that the investigator has to define the number of dipoles that better explains the variances of contributing sources a priori. In the standard N1/P2 dipole model for the analysis of the LDAEP two dipoles are

set in each hemisphere, one tangential dipole representing the PAC and one radial representing the secondary auditory cortex (Scherg & Von Cramon, 1985).

However, little effort has been made to specify if significant additional generators contribute to the scalp potential or account for an improvement of the residual variance in the dipole model. Electrophysiological studies conducted in the 1980s and 1990s already pointed out that the auditory N1 wave does not arise from a unitary source (i.e. auditory cortices) but reflects a superposition of sources with different functional significances (Giard et al., 1994; Knight, Hillyard, Woods, & Neville, 1980; Näätänen & Picton, 1987; Picton et al., 1999). Those studies were mostly based on derived sources from the scalp potentials or limited by a priori dipole models. Since then not much research has been done on the underlying mechanism of auditory processing during the N1/P2 time window, even though the methodology has improved significantly. For instance, better resolution in space and time can be achieved by using a few hundreds of highly sensitive magnetometer sensors in MEG or by utilizing a hybrid fMRI/EEG system which provides both, high spatial and temporal resolutions (Neuner et al., 2014).

Evidence from the recent studies comparing the diverging analysis methods in LDAEP research is in line with the assumptions that additional sources are activated. Hagenmuller et al. (2011) compared DSA analysis with single-electrode estimation within the same sample and found a significant difference between scores obtained with both methods. The authors assumed that a third source was additionally activated and contributed to the scalp signal captured by a single electrode. Actually, some studies used a third regional source in the dipole model in the frontal region, especially for high intensities in order to improve the residual variance between the modeled and the true signal recorded at the scalp (Hitz et al., 2011; Wyss et al., 2013). Surprisingly, a study by Mulert et al. (2002) compared DSA and LORETA for source localisation and did not find a significant correlation between the results of the two techniques. This dissociation could be explained because distinct types of source analysis methods were compared. DSA in contrast to LORETA is a discrete source analysis that requires a priori assumptions about the exact number of dipole sources based on physiological knowledge. In this study the authors used a two-dipole model instead of setting a third dipole. This could have led to a contamination of the true signal resulting from the tangential dipole activity representing the primary auditory cortex.

The present study aims at improving the analysis strategy of LDAEP investigations by taking into account all generators involved in loudness dependence by means of MEG analysis. We used wholehead MEG combined with a distributed source model approach which allows an exploratory analysis – without fixing the number of generators a priori – of the spatio-temporal profiles of the activated brain regions with excellent time resolution (Attal, Maess, Friederici, & David, 2012;

Ioannides, 2006; Ioannides, Poghosyan, Dammers, & Streit, 2004). The use of high-density recording by means of MEG/EEG has never been applied in the analysis of LDAEP thus far and the increased number of channels used in this study tends to result in a satisfactory disentanglement of the overlapping components in the brain (Dammers et al., 2007; Michel et al., 2004). We hypothesize that besides the auditory cortex additional sources contribute to late auditory evoked responses as observed from scalp recordings in the timeframe of N1m/P2m. We suppose that this interfering source activity is mostly apparent after high intensity tones due to the following reasons: First, while using dipole source models in the analysis of LDAEP an involvement of a third neuronal source was supposed to be present at particularly high intensities (Hitz et al., 2011; Wyss et al., 2013). Second, Näätänen and Picton (1987) already reported that an additional component was most easily observed at high intensities.

Our findings promise to be valuable in improving basic physiological knowledge of the involved processes in order to define prior assumptions in discrete source analysis, for instance to create an adequate dipole model (Scherg & Berg, 1991). Moreover, we intend to further elucidate the comparability of the various methodological approaches.

5.2.3 Methods

5.2.3.1 Subjects

Nineteen healthy male right-handed subjects participated in the study (mean age 26.5 ± 4.0 years). All volunteers were recruited from the staff of the Forschungszentrum Jülich and from a volunteer mailing list. Subjects that met the following criteria were excluded: current or prior history of neuropsychiatric disorders as assessed by the Mini International Neuropsychiatry Interview (M.I.N.I.) (De Pascalis, Cozzuto, & Russo, 2012); first-degree relatives with psychiatric disorders; drug or alcohol abuse; and smoking or a lifetime history of metabolic disorders. Subjects were instructed to consume neither alcohol nor any pharmaceuticals 48 h before or caffeine 12 h before measurements. Handedness was assessed by the Edinburgh Handedness Inventory (Oldfield, 1971). All subjects gave written informed consent. The study was approved by the Ethics Committee of the Medicine Faculty of the Rheinisch-Westfälischen Technischen Hochschule Aachen (RWTH Aachen University) and was carried out in accordance with the Declaration of Helsinki. One subject was excluded as not suitable for analysis because of poor signal quality from the MEG measurements which was most likely due to external noise.

5.2.3.2 Experimental procedure

Neuromagnetic field changes in response to auditory stimulation were recorded in a magnetically shielded room with a whole-head 248 magnetometer system (Magnes3600, 4D-Neuroimaging, San Diego, USA). Recordings were performed in a supine position with the subjects lay with their eyes open. During the passive listening of the auditory stimulation all subjects were asked to stay relaxed and to avoid movements. As attention to the auditory stimuli has been shown to modulate the auditory evoked potentials (Schechter & Buchsbaum, 1973), and therefore also the LDAEP (Baribeau & Laurent, 1987), a silent movie was shown to the subjects for the purposes of distraction and they were told not to pay attention to the auditory stimuli. The sinusoidal tones (1000 Hz, 40 ms duration with 10 ms rise and fall time) were generated by a digital signal processor (Multi I/O Processor RX8, TDT System 3, Tucker-Davis Technologies, Alachua, USA) and were presented binaurally through earphones with plastic tubes and ear plugs inserted into the outer ear canals. Two programmable attenuators for the left and right ear (PA5, TDT System 3, Tucker-Davis Technologies, Alachua, USA) were used to present the tones at 10, 20, 30, 40, 50 and 60 dB sensation level (SL) in a pseudo-randomized order with not more than two equal levels following each other and pseudo-random stimulus onset asynchrony between 2 and 3 s in steps of 17 ms. Individual hearing thresholds were determined prior to each experiment and calibrated over five times. The mean threshold across all subjects was approximately 20 dB sound pressure level (SPL) and was just above the level of system noise of 20 dB SPL (at octave-band around 1000 Hz) that means that the presented stimuli were in the range of 30 to 80 dB SPL. The individual threshold was examined to guarantee an equal perception of the tones at both ears and to control the variability due to differences in the stimulation settings. Moreover, it is important to dissociate between perceived loudness and physical sound intensity, as it is supposed that the activation in auditory cortex rather reflects the subjective perception (Langers, van Dijk, Schoenmaker, & Backes, 2007). A system specific constant time delay of 20 ms respective to the stimulus onset was taken into account and later subtracted for analysis.

5.2.3.3 MEG recording

The neuromagnetic activity was continuously recorded with a sampling rate of 678.17 Hz in a frequency range from DC up to 200 Hz. Prior to the MEG measurement, 5 head location coils were attached to subject's head. The position of the coils and the head itself was digitized using a 3D digitizer (Polhemus, 3space/Fastrack, Colchester, USA). Before and after each recording block, subject's head position was monitored by the head location coils, whereby a maximum difference of 5 mm for each experiment was accepted for further analysis. Eye movement and heart beats were

monitored simultaneously using electrocardiography and electrooculography, respectively (Brain Vision BrainAmp ExG MR, Brain Products, Gilching, Germany).

5.2.3.4 Individual anatomical MRIs

For the co-registration of the MEG coordinate frame with the individual brain anatomy, high-resolution T1-weighted MR-images were acquired for each subject with a voxel size of 1 x 1 x 1 mm^3 (3T, Trio, Siemens, Erlangen, Germany). The rendered head shape was matched to the surface of the scalp by means of customized software, providing an affine transformation matrix for co-registering the MEG head coordinate and the MRI coordinate system (Dammers et al., 2007). For MEG source space preparation the individual anatomical brain was extracted using Brain Extraction Tool (BET) as implemented in FSL (Version 5.0.4, FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) and a source space of approximately 10000-12000 nodes using an isometric 5 mm grid was defined prior to source analysis.

5.2.3.5 MEG signal processing

After acquisition, all data were band-pass filtered with a blackman windowed sinc filter (S. W. Smith, 1997) in the range of 1-200 Hz including notch filters at the power line frequency (50 Hz and the harmonics). Noisy MEG channels were excluded by visual inspection. Artifact rejection was automatically performed using the independent component analysis (ICA) as described in Dammers et al. (2008) . Artifact free epochs were extracted for each stimulus in a time window of ranging from -200 ms to +650 ms before and after stimulus onset, respectively. The epoch onsets were corrected for the time delay between generating and presenting the stimuli to the subject ears. A baseline correction was calculated from the pre-stimulus interval -200 ms to -50 ms. The first five epochs of each condition were excluded in order to reduce short-term habituation effects and epochs with threshold level above 3 pT were excluded by default. Within one subject, only the minimum number of accepted epochs (min. 70) was averaged according to the six conditions. Across subjects on average 73.8 (SD 1.3) out of 80 epochs were accepted for each condition and guaranteed an almost constant signal-to-noise ratio. Global field power (GFP) was calculated independently for each subject and condition and was normalized by dividing through the standard deviation of the baseline (Lehmann & Skrandies, 1980).

5.2.3.6 MEG source analysis

Magnetic field tomography (MFT; Ioannides et al., 1990) is a distributed source reconstruction

method for the localization of the primary current density as recorded by MEG. MFT belongs to the family of weighted minimum norm solver (Ioannides, 2006; Ioannides et al., 1995; Taylor, Ioannides, & Muller-Gartner, 1999). A detailed description of the algorithmic steps is described in (Ioannides, 1995). In short: Let m_i be the output of the *i*th magnetometer. Then the measurement m_i can be expressed as a linear functional of the primary current density $j(r)$ with the vector-valued lead field $\phi_i(r)$ describing the sensitivity profile of the *i*th sensor:

$$m_i = \int_Q \phi_i(r) \cdot j(r) \cdot d^3r \quad (1)$$

For estimating the current density $j(r)$ MFT is based on a probabilistic treatment of the inverse problem where the estimated current density is expressed as a linear combination of expansion functions:

$$j(r) = \sum_k^N A_k \cdot \phi_k(r) \cdot w(r) \quad (2)$$

with *N* being the number of detectors, $w(r)$ is a probability weighting function defined throughout the source space, incorporating any *a priori* information about source location, and A_k are expansion coefficients that can be determined from the measurement data. In contrast to the well-known MN approach, MFT uses a non-linear weighting function $w(r)$ that allows the weights to depend on the strength of the first solution $||j_0(r)||^p$ (with $p = 1$) of the primary current density (Taylor et al., 1999). The initial values for the weighting function in MFT is determined by training the algorithm using computer generated data before MFT is applied to the real data (Ioannides et al., 1990). During inversion the weight update $w_n = c \cdot w_0(r) \cdot \|j_{n-1}(r)\|^p$ is used to sharpen up the reconstruction within an iterative process, with w_0 being the initial weight and c being a constant to ensure that the sum of weights equals 1. In other words, the result of the first application is used to enhance the a priori probability weight in regions where the activity has been identified. The difference to MN estimation is that in MN based solver no iteration is involved (with $p = 0$) and $w(r) = w = 1$. Moreover, the regularization parameter in MN solvers is estimated only once (typically from the noise covariance matrix), while in MFT the parameter is adapted for each inversion (Ioannides et al., 1990). In this way MFT is computationally more demanding, but is able to reconstruct shallow as well as deep sources at a time (Y.-H. Chen et al., 2009; Dammers & Ioannides, 2000; Weidner, Boers, Mathiak, Dammers, & Fink, 2010).

Lead fields used (cf. Eq. 1) for the MFT analysis were computed from each individual subject with respect to a spherical head model. Current density source reconstruction was estimated based on the averaged MEG data. We used the modulus (strength) of the current density activation for further

analysis. The mean of the baseline was subtracted for baseline correction in each subject individually. Because we were only interested in calculating the root mean squared (RMS) values out of positive activations, negative values possibly resulting from baseline correction were set to zero. RMS values were calculated voxel-wise for these data for each subject in a sliding window of 50 ms latency with 25 ms overlap between 0 and 650 ms. In this way, full 3D reconstructions across time are provided with respect to the GFP distribution and the time characteristics of the auditory evoked components (Woods, 1995). These 4D spatio-temporal profiles (MFTrms values) were transformed into the Neuroimaging Informatics Technology Initiative (NIfTI) format using an interpolated common isometric voxel size of 2 mm^3 for both the MFTrms reconstruction and the MRI anatomical scans in order to combine the MFT solution with the FSL software. Each NIfTI-volume was then aligned to the MNI standard space (Montreal Neurological Institute, MNI152 2mm brain) using the non-linear registration algorithm (FNIRT, Non-linear Image Registration Tool; FSL). Finally, the standard space volumes were used for statistical analysis throughout.

5.2.3.7 Statistical analysis

For group analysis, the transformed MFTrms volumes for each sound pressure level and time window were entered into second-level statistical group analysis using the generalized linear model included in FSL. Monte Carlo Permutation was applied as a non-parametric measure with minimal need of assumptions about the data. Due to our particular interest in the highest sound levels, we performed one-sample permutation t-tests with 5000 permutations to assess brain activity differences in the processing of the highest (60 dB) vs. lowest (10 dB) sensation levels (Nichols & Holmes, 2002). The voxel-wise maps were thresholded using threshold-free cluster enhancement (TFCE; Smith and Nichols, 2009) at $P < .001$ at cluster-level for $T = 3.73$ and all significance values were family-wise error (FWE) corrected for multiple comparisons. Variance smoothing was applied using a Gaussian kernel of width 3 mm in order to increase the power of the test. Anatomical regions were defined by means of the Jülich Histological Atlas (JHA; Eickhoff, Heim, Zilles, & Amunts, 2006) at highest t-values and maximum probabilities of each anatomical label. For the regions that have not yet been defined in a cytoarchitectonic map, we used the macroscopic probabilistic Harvard-Oxford cortical structural atlas, provided with FSL. We performed a time-course analysis within the anatomical regions significantly activated in the t-test during the N1m time window for each condition to elucidate the temporal dynamics across regions. Based on the 4D spatio-temporal profiles of the MFT analysis we calculated RMS values in space by taking into account all voxels in each of these regions-of-interest (ROI) for each time point. The data was normalized by the maximum value found across all conditions in time for each subject respectively, thus providing comparisons between

subjects and conditions. In order to statistically evaluate the best fit lines (linear or quadratic) of the slopes generated from mean RMS activations versus intensity levels (Fig. 8) across the ROIs, we used hierarchical linear regression analysis (SPSS version 22 for Mac) using RMS values as the dependent measure.

5.2.4 Results

5.2.4.1 Source analysis

Figure 6 shows the GFP over 248 magnetometers of the averaged auditory evoked field calculated across all sound pressure levels. Based on these latency peaks we concentrated on the activity in the following time windows 75–125 ms for N1m and 175–225 ms for P2m.

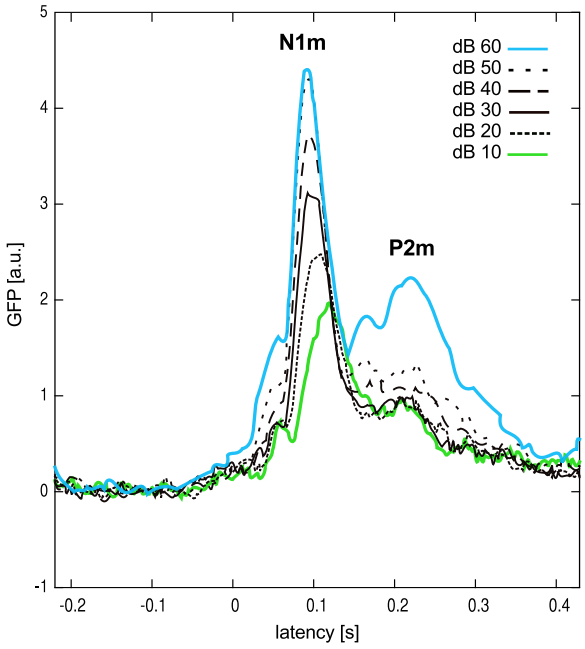

Fig. 6. Group averages (n=19) of global field power (GFP) after auditory stimuli presentation (10-60 dB SL). The N1m peak occurred between 75-125 ms, the P2m peak between 175-225 ms. MFT analysis were conducted within these time windows. Reference level of intensity is SL.

In the N1m time window we found significant neural activations in the primary auditory cortex (PAC), the posterior cingulate cortex (PCC), the premotor cortex (PMC) and the primary somatosensory cortex (Fig. 7A). Moreover, activation was found in the paracingulate cortex. In the time window of P2m significant activations were shown in the PAC, the primary and secondary visual cortices extending into the precuneus and the PMC (Fig. 7C). Table 5 provides the t-contrast values and MNI coordinates.

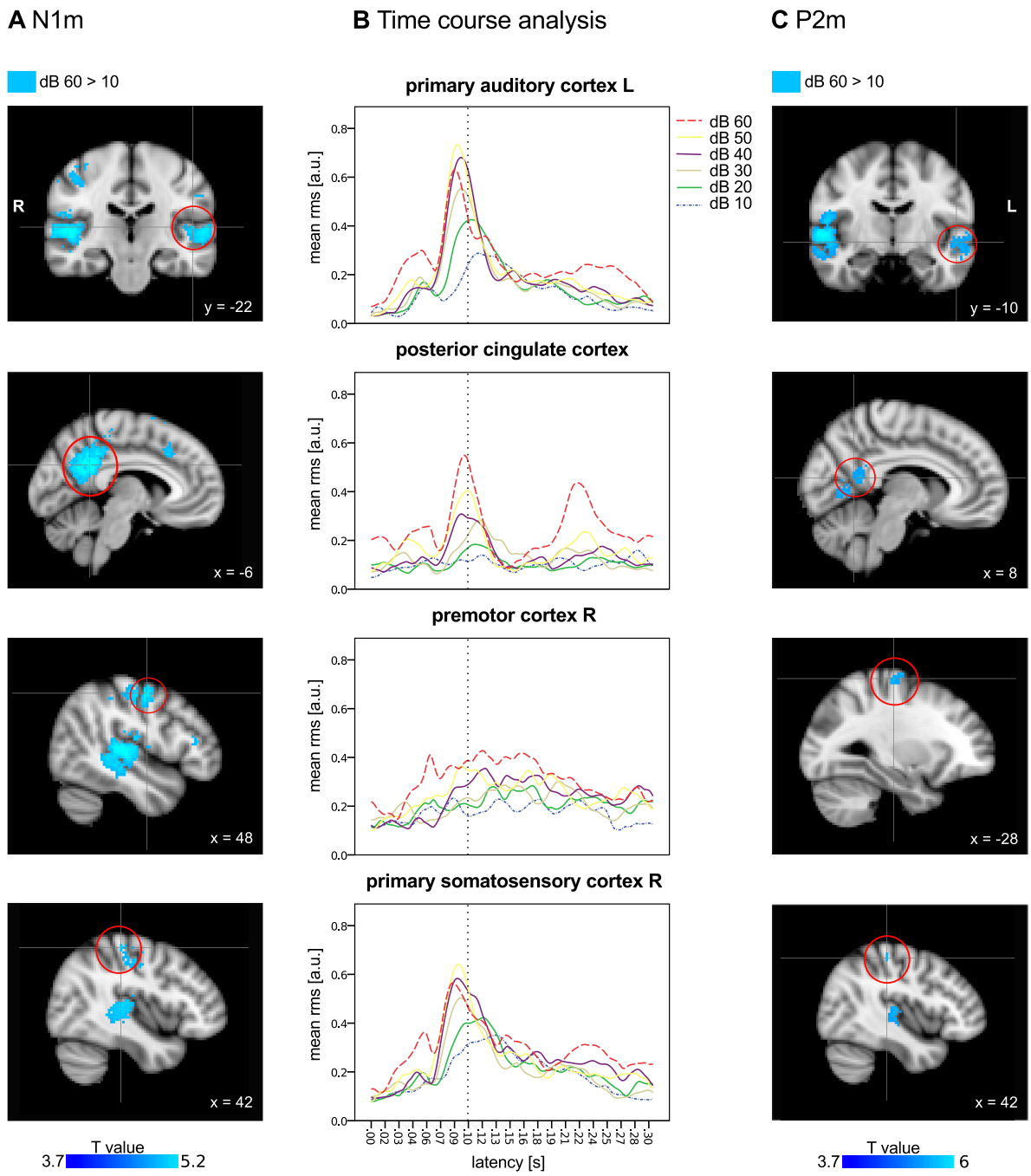

Fig. 7. MEG activation for the contrast analysis between the highest and the lowest intensity in the time window of N1m (A) and P2m (C) (N = 19, thresholded below P < .001 at cluster level for T = 3.73, FWE-corrected). Time course analysis between 0 and 300 ms poststimulus in each ROI (B). Vertical dotted line indicates the latency of N1. L: left hemisphere; R: right hemisphere. RMS values for time courses were low pass filtered at 30 Hz for graphical reasons. Reference level of intensity is SL.

Table 5. Anatomical locations of all significant T-contrast activations in MEG magnetic field tomography in the time window of N1m and P2m.

	Region	L/R	k	t-value	MNI coordinates		
					x	y	z
	60−10dB						
N1m	Primary auditory cortex/Planum temporale	R	2173	5.58	50	−26	6
	Primary auditory cortex/Middle temporal gyrus	L	800	4.58	−50	−22	8
	Posterior cingulate cortex	L	2173	5.7	−6	−50	28
	Posterior cingulate cortex/Precuneus	R	2173	5.4	4	−48	30
	Premotor cortex/Primary somatosensory cortex	R	368	5.16	48	−6	50
	Paracingulate cortex	L	95	4.46	−4	20	44
P2m	Primary auditory cortex	R	1651	6.42	52	−16	2
	Primary auditory cortex/Middle temporal gyrus	L	385	4.34	−52	−10	−6
	Visual Cortex V1 and V2/Precuneus	R	212	3.85	8	−56	14
	Premotor Cortex	L	117	4.59	−28	−20	66

N = 19, thresholded below P < .001 at cluster level for T = 3.73, FWE-corrected. To keep apart the different clusters, local maxima within 10 mm of each other in each cluster were defined. L: left hemisphere; R: right hemisphere; k: cluster size; MNI: Montreal Neurological Institute (mm).

5.2.4.2 Time course analysis of the activation in a ROI

The results of the time course analysis revealed different activation pattern among the ROIs between 0 and 300 ms (Fig. 7B). In the sensory cortices a saturation effect of the N1m could be observed that means that the highest sound level did not evoke a higher mean MFTrms value. According to latencies, the N1m component peaked between 88 and 133 ms across all intensity levels in the sensory cortices (auditory and somatosensory) and the PCC (Table 6). The time courses of the mean MFTrms values in the PMC did not show accentuated peaks. However, a loudness dependency is still obvious and a maximum value at 119 ms for the 60 dB condition was reached.

Relating to the P2m component a distinct peak with the highest sound level is shown in the PCC. In the remaining ROIs the peaks were rather broad and flat, nevertheless showing an increase around 220 ms in the sensory areas whereas no clear peak was observed in the PMC.

The temporal sequence of activations for the highest sound pressure levels determined from the N1-peak latencies was from PAC and primary somatosensory cortex to PCC and finally to PMC (Fig. 7B, Table 6). The left and the right PAC were activated simultaneously.

Table 6. N1-peak latencies for each sound pressure level within ROIs.

Region	L/R	dB SL					
		10	20	30	40	50	60
		ms					
Primary auditory cortex/Planum temporale	R	114	107	96	96	92	89
Primary auditory cortex/Middle temporal gyrus	L	111	101	98	96	92	90
Posterior cingulate cortex/Precuneus	L/R	117	111	117	96	104	99
Premotor cortex	R	88	172	200	121	96	119
Primary somatosensory cortex	R	133	120	95	92	93	88

L: left hemisphere; R: right hemisphere.

5.2.4.3 LDAEP slope differences among the ROIs

A supplemental research question arose during our analysis based on the observation of the saturation effect. Therefore, the slope of the loudness dependence of the RMS activation across all intensities was generated at the previously defined time window of N1m (75-125 ms) within each ROI.

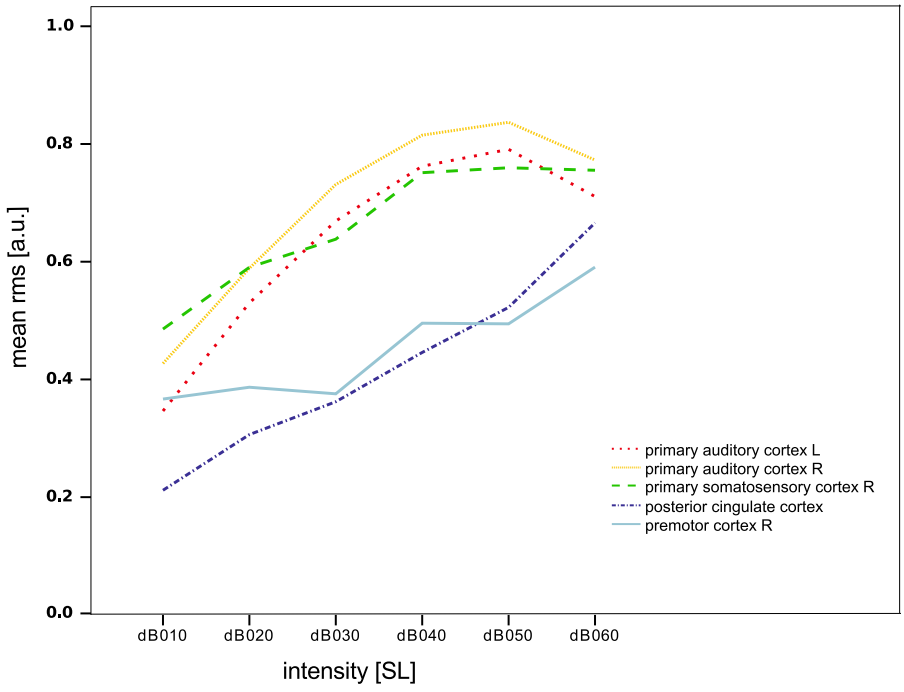

Fig. 8: ROI analysis of average current densities at N1m over all intensities. L: left hemisphere; R: right hemisphere.

Figure 8 shows that the mean MFTrms value in the PAC increases steadily with lower intensities but decreases with the highest intensity in both hemispheres. A similar trend was observed for the activation in the primary somatosensory cortex. On the other hand, the activation in the PCC and the PMC steadily increases with sound pressure level.

These characteristics were confirmed by hierarchical linear regression analysis. Analysis revealed that a quadratic model was able to account for an additional amount of total variance of a linear model alone in the left and right PAC (left: $\Delta R^2 = .096$, $P = .000$; right: $\Delta R^2 = .083$, $P = 0.000$). No additional variances were explained for the quadratic model in the PCC ($\Delta R^2 = .003$, $P = .531$), PMC ($\Delta R^2 = .006$, $P = .378$) and in the primary somatosensory cortex ($\Delta R^2 = .018$, $P = .122$).

5.2.5 Discussion

This study assessed the neuroanatomical correlates of the N1m/P2m-complex evoked by the presentation of brief tones of different intensities. In addition to prior studies, multichannel MEG was used and source reconstruction was performed using a data-driven approach. Brain activation during the processing of the tones presented with the highest sound pressure levels compared to those with the lowest sound pressure levels was computed. However, the waveforms of both the N1m and P2m are not generated by a single region, but they rather reflect the sum of several relatively independent latent components generated by a distributed network involving PAC, primary somatosensory cortex, motor cortex and the PCC.

5.2.5.1 Sources in and near the auditory cortex

A large number of studies examined brain activity generated by loudness variation of auditory stimulation with EEG (Hegerl et al., 1994; Neukirch et al., 2002), MEG (Ioannides, 2006; Vasama, Mäkelä, Tissari, & Hämäläinen, 1995) or fMRI (Jäncke et al., 1998; Mulert et al., 2005; Wood et al., 1984). They have consistently found sources in and in the vicinity of the auditory cortex and further investigated the underlying neural mechanisms (i.e. increased responses or spatial extent of activated cortex volume) by intensity change. In line with these studies we found activation in the primary auditory cortex and specifically as shown in other MEG studies (Godey, Schwartz, De Graaf, Chauvel, & Liegeois-Chauvel, 2001) the activation extended into the planum temporale at the N1m time-range (Table 5, Fig. 7). In contrast, the activation showed a slight posterior to anterior shift toward the planum polare in the time window of the P2m.

5.2.5.2 Contribution of neural activity outside the auditory cortex

To our knowledge there is very little research reporting on sources outside the auditory cortex underlying the LDAEP. It is known that subcortical structures involved in the early processing of auditory stimuli such as the superior olive, the inferior colliculus and the medial geniculate body are sensitive to noise levels, showing a higher activity to increasing levels (W. T. Roth et al., 1980).

Nevertheless, references from studies describing the generators of the auditory N1 and P2 components are extensive and should be taken into account for the interpretation of our results. Näätänen and Picton (1987) already pointed out that not only one predominant generator but also other sources contribute to the scalp recorded N1 peak in auditory processing. These authors proposed a third component underlying the N1 wave often referred as the "unspecific component" whose exact location is still unknown, but is proposed to lie within the frontal motor cortex and PMC in the precentral gyrus. Justification for these assumptions came from the effects of interstimulus intervals (ISI) on the scalp topography of the N1, as refractoriness processes become more active during increased ISI (>4 s) and thus lead to less specific additional sources near the vertex (Hari et al., 1982; Näätänen & Picton, 1987; Velasco & Velasco, 1986). Moreover, intracerebral recordings in monkeys supported additional generators of auditory evoked potentials in the PMC and motor cortex (Arezzo, Pickoff, & Vaughan, 1975). Since then only few studies have contributed to the clarification of this third component by means of scalp current density and dipole analysis. The characteristics of this frontal response, however, are still ambiguous. Some authors (Alcaini, Giard, Thevenet, & Pernier, 1994; Giard et al., 1994; Näätänen & Picton, 1987) described a component with contributions from sources outside the auditory cortex with a peak latency of 100 ms, most easily recorded at intensities greater than 60dB SPL and with long as well as short ISIs. The sources were described to lie within the posterior frontal lobe in the motor cortex (BA4), the supplementary motor area (BA6) or the cingulate gyrus. Other authors reported a component generated by the same regions but at later latencies (i.e. around. 125 ms; 140 ms) or with a longer refractory period, that is emerging only with longer ISIs (Alcaini et al., 1994; Picton et al., 1999).

Our study is the first using a distributed source model to show an additional significant activation in the PMC during the N1m time window. The response remained relatively constant between 60 and 180 ms and peaked at 119 ms in the condition of the highest intensity (Fig. 7B). These findings are in agreement with the findings of the aforementioned previous work. The activation in the PMC might be related to the acoustic startle response to a sudden loud noise and is viewed as an aversive response to novel and potentially harmful stimuli (De Pascalis et al., 2012; P. J. Lang, Bradley, & Cuthbert, 1990). The primary somatosensory cortex that is additionally activated in the present study could also play a considerable role in the processing of the startle response (Neuner et al., 2010).

Other authors pointed out that the supplementary and premotor areas may be related to the orienting response, an orientation of attention to a change in the environment and linked to the planning and execution of motor responses even though the subjects had no such task to perform (Hari et al., 1982; Y. H. Kim et al., 1999; Näätänen & Picton, 1987).

Furthermore, the PCC was observed to belong likewise to the network of auditory intensity processing. The results show a greater increase in activation with higher sound pressure levels in the time window of N1m and P2m. Interestingly, one single LDAEP-study using PET reported activation in this region (Lockwood et al., 1999), but with maximal activation at lower intensities. The authors assigned to this region a significant role in regulating or controlling the magnitude of intensity and also mentioned a possible link to the attention system. However, the functional role of the PCC is currently not clear and competing theories exist (Leech & Sharp, 2014). According to our findings (Fig. 7B) the PCC is activated after the primary sensory areas and may subserve evaluative functions such as monitoring sensory events and homeostatic processes (Vogt, Finch, & Olson, 1992). In fact, the PCC shows no direct innervations to primary sensory and motor areas (Parvizi, Van Hoesen, Buckwalter, & Damasio, 2006), but connections to the prefrontal cortex. Interestingly, the PCC is modulated by serotonergic neurotransmission (Hahn et al., 2012). Long term treatment with SSRIs were reported to have a significant effect on the neuronal structure (Kraus et al., 2014) and function (Matthews et al., 2010) of the PCC. Moreover, transmitter receptor fingerprints of the cerebral cortex indicate that the mean densities of 5-HT_{1A} and 5-HT_2 receptors are comparable between PCC and PAC (Zilles et al., 2002). While studies about treatment response prediction are promising in LDAEP research, the optimal used analysis method is still debated (Jaworska et al., 2013; Mulert et al., 2002; Park et al., 2011). However, scalp measured potentials, for instance at Cz, might reflect serotonergic modulated responses from both the PAC and the PCC (e.g. Linka et al., 2004). To our knowledge, there exists no study investigating a modulatory effect of the 5-HT system on the PMC.

Another interesting finding is that the presentation of auditory stimuli activated not only the primary auditory cortex but also the primary visual and somatosensory cortex. This is in agreement with the theory of multisensory integration that states low level integration in different sensory modalities (i.e. auditory, visual and somatosensory) and controverts the traditional assumption that multisensory integration is a higher order process (Schroeder & Foxe, 2005).

By comparing the activation pattern between the time windows of N1m and P2m, we did not find any significant differences. Figure 7 reveals that there has been a slight shift of activation from the PCC toward the precuneus and the visual cortices.

5.2.5.3 LDAEP slope differences among the ROIs

Our data showed that the characteristics of responses to high intensity levels differ between the ROIs. Responses at the highest intensities in the PAC level off, what is commonly called saturation, whereas the slope in the PCC and PMC continuously increases along the intensity level (Fig. 8). Saturation is particularly susceptible to varying methodological conditions and has been documented when stimuli were presented at shorter intervals than 2.5 s or when the intensity was held constant within blocks (Näätänen & Picton, 1987). However, this effect is discussed controversially to occur in the auditory cortex. While some studies reported saturation effects (Bruneau et al., 1985; Lockwood et al., 1999; Neukirch et al., 2002; Reite, Zimmerman, Edrich, & Zimmerman, 1982) others did not (Brechmann et al., 2002; Calvert et al., 2001; Hall et al., 2001; Jäncke et al., 1998; Langers et al., 2007; Wood et al., 1984). Thus it appears that the mechanism leading to saturation is not well understood. The hypothesis of intensity selectivity of neurons contribute to sorting out this issue: first, the observation of spatial extend of activated voxels with increasing intensities in fMRI leads to the hypothesis of an increase in the number of responding neurons. As a consequence, involved neurons spread to auditory association areas when the monotonic neurons in PAC are saturated (Jäncke et al., 1998; Uppenkamp & Roehl, 2013). The observed response saturation in PAC in our data might be a consequence of this restricted response. Second, it is supposed that neurons in the auditory cortex have specific functions related to the decomposition of acoustic information (Seifritz et al., 2002). The so called monotonic and non-monotonic intensity tuned neurons differ in their response to intensity level to protect the sensory system from overstimulation and are thought to be topographically organized without forming a clear intensity map in PAC (Woods et al., 2010). An incoming sensory stimulus gets processed in these neurons by a cortical intensity tuning mechanism that is based on the basic principle of the canonical microcircuit. This process emphasizes that temporal interaction between excitatory thalamic and inhibitory input via interneurons is pivotal for intensity tuning (Ojima, 2011; Wu, Tao, & Zhang, 2011). The fact that inhibition is less represented than excitation by the BOLD activation in functional imaging (Waldvogel et al., 2000), having in mind this interactional process, this could explain why most fMRI studies investigating response saturation in PAC did not find a saturation effect.

Interestingly, the characteristics of the activation found in the PMC in our data is in line with earlier observations from our working group: Hagenmuller et al. (2011) revealed that the LDAEP slopes (amplitudes plotted against intensity) differentiate between DSA and single-electrode estimation at Cz. While the slope induced at the scalp showed a steady rise to increasing intensity levels, the potentials within PAC saturated with the highest intensity. This effect is also shown in the present data, when activations over intensities in PAC and PMC are compared. Considering the similar

LDAEP slopes at Cz and within the PMC, we can suppose that the single-electrode method's sensitivity to potential changes outside the temporal cortex is affected by this frontal source.

Furthermore, the nature of synchronicity of the activated brain regions plays an important role. Huang et al. (2003) reported a varying percentage of explained variance at the Cz electrode from bilateral superior temporal gyrus (STG) sources between healthy controls and patients with schizophrenia. The authors attributed this group differences to an additional generator outside the STG that is supposed to fire synchronously or asynchronously with the sources in auditory cortex. In the case of synchronously firing – as showed in healthy subjects – it is possible that the variances of both sources were correlated and therefore not detectable at Cz. Interfering contributions from extra-auditory areas, for instance from the PCC, could lead to additional variances of the results across psychiatric disorders complicating the use of different analysis approaches (Jaworska et al., 2013; Park et al., 2011).

Substantive procedural differences make direct comparisons between studies on LDAEP difficult. As discussed by many authors, the stimuli presented to the subject have a crucial impact on the resulting signal and its sources. For example there is a big range of intensity levels presented in LDAEP studies – varying from two to six sound levels – what implicates differences in initial levels that in turn have an impact on the N1m amplitude (Park et al., 2011; Uhl et al., 2011). Moreover, as already discussed above the ISI is supposed to have an effect on the appearance of the sources because recovery cycles differ between populations of neurons (Coch, Skendzel, & Neville, 2005). It has even been demonstrated that the way the intervals are presented (randomized or in a block) influences the signal (Zacharias et al., 2012). In our study we did not focus on variation in ISI and its specific effects on source localisation remain open. Furthermore, an effect of frequency on the loudness dependence was reported (Calvert et al., 2001).

Further research using dipole source analysis that takes this prior knowledge about the generating sources into account, will need to be undertaken. Sophisticated Bayesian inversion schemes (Kiebel, Daunizeau, Phillips, & Friston, 2008) are useful to objectively compare competing dipole models that vary in the numbers of dipoles or other informative priors and decide which theory explains the observed data best.

5.2.5.4 Conclusion

This study concentrates on the underlying mechanism of activation during the processing of auditory evoked fields related to the variation of tone intensities. Our results indicate that apart from the auditory cortex and its association areas other regions are activated post stimulus in the time window of N1m and P2m. The most striking result to emerge from the data is additional activation in the

premotor (PMC) and primary somatosensory areas with the highest intensity levels. Moreover, we found loudness dependent activation in the posterior cingulate cortex (PCC). For further investigation we analyzed time courses of the activity and receptiveness to rising intensity levels in these areas. The motor response might originate from a reaction (e.g. attentional, orienting or protective) of the organism to exceptional stimuli and is most likely indicated at the scalp level near vertex. As a result, source localization and single-electrode estimation do not cover the same sources and we suggest that these methods are not directly comparable in the analysis of LDAEP.

6. General discussion

The LDAEP paradigm has been highly influential in psychiatric research as a potential non-invasive biomarker for central serotonergic activity (O'Neill, Croft, et al., 2008). Concomitantly, it has been used to make predictions of serotonergic drug response in major depression (Hegerl et al., 2001; Leuchter et al., 2009; Park & Lee, 2013). The present dissertation aims to investigate the LDAEP both in a clinical sample and in healthy subjects with special regard to the problems that exist when using imaging biomarkers. It is essential to address present limitations in order to improve the validity of the LDAEP and advance its application in clinical practice.

The research questions raised in this thesis were motivated by the following shortcomings: first, when patients with schizophrenia are investigated, they may have only one or two symptoms in common, which is why potential alterations in serotonergic levels as assessed by the LDAEP cannot be attributed to the same biological roots. Second, although the LDAEP is a strategy that is very common in psychiatric research, a standard protocol for its use is missing. Recent studies showed that results obtained with different source analysis approaches are not comparable (Hagenmuller et al., 2011; Jaworska et al., 2013; Mulert et al., 2002). This inconcistency may result from the use of different methodologies that represent the neural activity of distinct underlying neural generators.

The aim of the first study (chapter 5.1) was to refer the LDAEP to dimensional measures of mental functions in order to avoid the problem of heterogeneity that characterizes schizophrenia. We therefore examined patients with schizophrenia, who had been scaled with a standardized questionnaire that determined the severity of different syndromes. Serotonergic dysfunction, as measured by the LDAEP, was shown to differ within the phenotypic definition. The second study (chapter 5.2) used a distributed source analysis method to shed light on the neurobiological mechanisms of loudness dependence in healthy subjects. The findings revealed that a distributed network of underlying neural generators is involved. Furthermore, there may be a decisive influence on results obtained from scalp channel measures.

In the following, the findings of the two empirical studies will shortly be summarized and discussed in a broader context by taking a more overarching perspective than was outlined in the respective discussion sections of the particular studies.

6.1 LDAEP as a biomarker in schizophrenia research

Schizophrenia is a complex and multifactorial disorder with a heterogeneous manifestation of psychopathological symptoms. The unravelling of its exact neuronal origins is challenging. The present study aimed to examine the underlying chemical disturbances in negative symptoms, a subgroup of symptoms within the diagnosis of schizophrenia, by using LDAEP.

Accordant with our hypothesis, patients with predominant negative symptoms showed a significantly stronger LDAEP and respectively lower serotonergic activity than controls. Consistent with earlier findings in schizophrenia (Schooler, Buchsbaum, & Carpenter Jr, 1976), our results may be interpreted as a failure of the sensory system to sufficiently control for stimulus intensity (strong LDAEP) in the chronic state of schizophrenia. This may be interpreted as a lack of protection against overstimulation. Such a failure in modulation of sensory input might be controlled by both a cortical intensity tuning mechanism in the PAC (Wu et al., 2011) and by higher cognitive functions that orientate attention or monitor sensory events (Näätänen & Picton, 1987; Wyss et al., 2014). On the other hand, based on the assumption of an inverse relationship between LDAEP and 5-HT functioning (Juckel, 2005), this finding points to a reduced serotonergic activity. The prevalent theory of the aetiology of negative symptoms suggests a decreased serotonergic and dopaminergic activity in the mesocortical system (Abi-Dargham, 2007; B. Roth & Meltzer, 2000). Serotonin has a modulating effect on the dopaminergic system. However, to date it is not clear whether this regulation is excitatory or inhibitory. The modulatory effect probably depends on the specific 5-HT receptor types that are involved (Boureau & Dayan, 2010) as well as mediating effects through interneurons (Kapur & Remington, 1996). Moreover, this opposing effect is reflected in drug action that improves the outcome of negative symptoms in schizophrenia on the one hand via 5-HT_2 antagonism (i.e. atypical neuroleptics) and on the other hand via enhancing serotonergic levels through serotonin reuptake inhibition (i.e. SSRI) (Leucht et al., 2011; Silver, 2004; S. P. Singh et al., 2010). In future studies the exact mechanisms underlying the serotonin-dopamine interaction and its relevance to negative symptoms as well as the action of pharmacological agents in the treatment of these symptoms have still to be elucidated (De Bartolomeis, Buonaguro, & Iasevoli, 2013; B. Roth & Meltzer, 2000).

Contrary to our findings, the majority of studies reported weaker LDAEP (stronger serotonergic activity) in patients with schizophrenia diagnosed on the basis of current diagnostic categories (Gudlowski et al., 2009; Juckel et al., 2003; Juckel, Gudlowski, et al., 2008; Park et al., 2010). Some of these studies also stated weak correlations between LDAEP values and psychopathological scales, but the findings were inconsistent (Gudlowski et al., 2009; Juckel, Gudlowski, et al., 2008; Ostermann et al., 2012). However, these results should be treated with caution because reflecting a mixture of symptoms based on different underlying biological processes, they may be biased.

Additionally, the methodological parameters used in these studies are hardly comparable and beyond that, the caveats outlined in chapter 2.2 may have influenced the results (e.g. gender, analysis approach).

Regarding the present findings the following conclusions can be drawn:

On the one hand, the study supports the LDAEP as valid tool for identifying differential clinical features of schizophrenia by reflecting its aetiological factors, i.e. dysfunctional serotonergic activity. Due to the pivotal role that serotonin and dopamine play in the generation of negative symptoms in schizophrenia, we extracted, supplementary to the present study, deoxyribonucleic acid (DNA) from whole blood samples in order to learn more about the genetic causes of the illness. By examining fifteen subjects (controls, n=7; patients with schizophrenia, n=8), we focused on the *COMT*-gene and the 5-HTTLPR being a part of the serotonin transporter (*SERT)*-gene. COMT is the enzymatic product of the *COMT*-gene and influences dopamine levels by degrading of synaptic dopamine and the SERT is responsible in the reuptake of serotonin in the presynaptic neuron (Cooper & Roth, 2003). The results provided a significant association between the *COMT* polymorphism and the LDAEP in accordance with earlier observations (Juckel, Kawohl, et al., 2008). The main conclusion of this finding is that neurotransmitters other than serotonin may have an influence on the LDAEP, and this is currently a matter of investigation as further discussed in chapter 6.3. However, due to the small sample sizes in the defined groups of allele carriers (n=2/13) and genotypes (n=2/8) (see table 1 and 2 in the appendix) the results should be interpreted with caution. They were therefore not published in the enclosed paper. Further research is needed to clarify the aetiology of positive and negative symptoms in more detail before the LDAEP can be used as a diagnostic tool in clinical work. It is still unresolved which of the neurotransmitters, serotonin or dopamine, is the driving force when it comes to a functional abnormality (Abi-Dargham, 2007; Goff & Evins, 1998; Winograd-Gurvich et al., 2006).

On the other hand, as outlined in the introduction of this thesis, the aetiological heterogeneity of clinical diagnosis is only one of the problems occurring within the scope of clinical biomarkers. Another important aspect that should be considered is that contradictory findings may result from different methodologies used in the parameterization of the LDAEP (Hagenmuller et al., 2011). In this context, an interesting finding from our study was that the LDAEP at Cz revealed no significant differences between the groups. Thus, the estimation at a single electrode site was confirmed to be not comparable with dipole source analysis. This accords with earlier observations in which both methodologies were compared and significantly different results were found (Hagenmuller et al., 2011). A possible explanation is that an additional source outside the auditory cortex might be active during auditory processing and contribute to the electrical potentials measured at the scalp surface

(Alcaini et al., 1994; Arezzo et al., 1975; Giard et al., 1994; Näätänen & Picton, 1987; Picton et al., 1999). Crucially, the activity from such an additional generator might be either synchronized or asynchronized with the activity in the auditory cortex depending on the pathological state of the sample examined (Huang et al., 2003). In other words, the mixture of activity from different sources that is measured at a single electrode, for example Cz, might differ between patients with schizophrenia and healthy individuals. With due consideration of these arguments it may not be surprising that studies investigating the LDAEP in schizophrenic patients reported controversial results, as different analysis methodologies were used across studies.

The study has certain limitations. One source of weakness in this study, which could have affected the reported results was that all patients were under medical treatment. The increased loudness dependency in patients could be caused by the administered antipsychotic therapy (i.e. the EPSP might be enhanced by a reduced inhibitory effect through GABAergic interneurons which in turn receive a reduced input via 5-HT_{2A} receptors, because they are blocked by atypical neuroleptics) (Meltzer, 1999). Such an effect of medication is reflected by a study that investigated patients with schizophrenia treated with atypical neuroleptics over four weeks (Juckel et al., 2003). The results showed an increased LDAEP after the treatment compared to baseline measurements. More information on the relation between chronically modulating effects of the serotonergic system and the LDAEP by means of longitudinal studies would help us to clarify this matter (Meltzer & Massey, 2011).

Another limitation of this study is that the numbers of patients and controls were relatively small. However, it must be mentioned that the effect sizes that quantify the size of the difference between two groups were rather large (Cohen's d between 1.04-1.20). Further studies should compare the LDAEP within schizophrenia by reflecting both the negative and positive symptom clusters, however a larger sample size is needed to guarantee a broad variance in symptom characteristics.

An issue that was not addressed in this study was the determination of the individual hearing levels. Based on the original research by Hegerl and Juckel (1993), in which they used sound pressure levels, most ensuing studies retained this reference. However, the estimated prevalence of hearing impairments, implying that the hearing level lies above 35 dB in the better ear, is between 10-12% in general population (age > 15 years) (Stevens et al., 2013). Therefore, the presentation of stimuli of different intensities should be referenced to an individual's hearing threshold. In the second study presented in this thesis we accounted for this paucity by determining the SL prior to each experiment. In addition, because we used plastic tubes in the MEG experiment to deliver the tones, this procedure allowed us to control for further variability due to stimulation setting.

The questions brought up in study I relating to the lack of comparability of different analysis approaches were further investigated in study II. There we aimed to unravel the generators that are involved in the LDAEP paradigm, particularly during loudness perception by means of MEG.

6.2 Generating sources of LDAEP

The aim of study II was to explore the neural activations in the time course of the LDAEP in order to understand why the commonly used analysis strategies (i.e. dipole source analysis and single electrode estimation) are not comparable (Hagenmuller et al., 2011). The study yields the possibility of giving researchers using LDAEP strong arguments for deciding on an analysis strategy that is the most specific for measuring activity in the PAC. Moreover, referring to the problems about the validation of biomarkers as outlined in the introduction, the unravelling of the underlying sources might improve the construct validity of the LDAEP and support the interpretation of its mechanisms.

Several studies have been dedicated to investigating the underlying neuronal mechanism of auditory intensity processing by means of electrophysiological methods (Alcaini et al., 1994; Budd et al., 1998; Hari et al., 1982; Näätänen & Picton, 1987; Velasco & Velasco, 1986). So far, only assumptions about an additional source being involved have been drawn in the respective studies and the results are ambiguous. However, none of these studies used distributed source analysis to explore the underlying generators. We therefore investigated 19 healthy male subjects using MEG to shed light on the LDAEP's generators and its dynamics over time. MEG was chosen because of its advantages compared to EEG and fMRI as outlined in chapter 4 and 4.3 respectively. Specifically, physiological knowledge based on fMRI studies might be problematic when transferred to EEG analysis for setting the number of dipoles a priori (Michel et al., 2004), because the hemodynamic response measured with fMRI only reflects the underlying neural activity indirectly (Logothetis, Pauls, Augath, Trinath, & Oeltermann, 2001). However, we used MEG to specifically address this research question and not to present an alternative measurment method to EEG, especially since MEG scanners are very rare and the costs are too high for clinical application.

The results revealed that during the processing of tones with high intensity levels in the time window of N1/P2, not only auditory association areas were activated, but also additional brain regions, namely the somatosensory cortex, the premotor cortex (PMC) and the posterior cingulate cortex (PCC).

Of special interest is the activation in the PCC, where strong loudness dependence was observed. Interestingly, the PCC contains a mean density (i.e. averaged over all cortical layers) of serotonergic receptors (5-HT_{1A} and 5-HT_2) that is comparable with that in the PAC. This was shown by the seminal study of Zilles et al. (2002) that integrated data from different imaging modalities to produce

transmitter receptor fingerprints in the human cerebral cortex. A human PET study additionally revealed that 5-HT_{1A} receptors were responsible for a modulatory effect on the key region of the default mode network (i.e. the PCC is a pivotal hub in the human resting brain with the highest number of functional connections) (Hahn et al., 2012). Crucially, the PCCs structure (Kraus et al., 2014) and function (Matthews et al., 2010) was reported to be altered by chronic SSRI pharmacotherapy. It can thus be concluded that by analysing scalp potentials in the context of the LDAEP, the contributing signal from the PCC could likewise add a significant input. Under consideration that the LDAEP is traditionally computed from the activity either extracted from dipole source analysis or measured at scalp electrodes, these findings provide evidence that the comparability between these two methodologies is problematic. They may not represent activity from the same sources.

These conclusions might have important implications for researching treatment response prediction. Studies that examined, in a comparative manner, whether the predictions made about favourable response to SSRI treatment were relying on the analysis strategy used for the LDAEP showed inconsistent findings. One study favoured the use of source analysis (LORETA) compared to scalp channel measures to achieve the best scores in discriminating responders from non-responders (Jaworska et al., 2013), which may well be true when using dipole source analysis (Mulert et al., 2002). An other study found that the power of LORETA as an analytical tool is comparable to that of scalp measured LDAEP (Park et al., 2011). And some other reports supported satisfying response prediction to SSRIs by measurements at single electrodes only (T. W. Lee, Yu, Chen, & Tsai, 2005; Linka et al., 2004). However, the sample under investigation varied between patients with major depression and anxiety disorder. The involvement of non-specific brain areas, as found in our study, may provide an alternative explanation as to why the above-mentioned studies found favourable prediction for treatment response by means of scalp channel measures. Moreover, due to the additional contribution from non-specific brain areas where synchronized activity can have an interactive effect on the variation of the result, the findings may also differ across psychiatric disorders (Huang et al., 2003).

Another line of argument that our data indicates is that the characteristic responses to increasing intensity, especially responses to the highest intensities, may reflect differences in cortical origin. Indeed, when plotting the amplitude against intensity, the slopes differ across the regions of interest. The primary auditory cortex showed decreased amplitudes at the highest intensities, in primary somatosensory cortex a similar tendency was observed, whereas the PCC and PMC revealed a linearly increasing slope. We assumed that these characteristics in the primary sensory areas were attributed to a so-called saturation effect, allowing the sensory system to protect itself from over-stimulation. However, the existence of such an effect in the auditory cortex is discussed with controversy across several different imaging methods (Bruneau et al., 1985; Calvert et al., 2001; Hall

et al., 2001; Jäncke et al., 1998; Langers et al., 2007; Lockwood et al., 1999; Neukirch et al., 2002; Reite et al., 1982). Accordingly, different theories exist about its underlying mechanisms (Jäncke et al., 1998; Ojima, 2011; Uppenkamp & Roehl, 2013; Wu et al., 2011). Notwithstanding, these observations are of great importance as they focus on improving the analysis strategy in LDAEP research, since exactly the same pattern can be recognized when LDAEP slopes are either plotted from the Cz electrode or from dipole source analysis displaying the activity of the PAC (Hagenmuller et al., 2011). In this respect, activity recorded at Cz may include activity generated in the PCC and PMC. Interestingly, in investigating the augmenting/reducing phenomenon it was noted that the response patterns differed within the same individuals depending on the placement of the electrode (i.e. midline or temporal) (Carrillo-de-la-Pena, 1999; Prescott, Connolly, & Gruzelier, 1984). As a consequence, the comparability between results obtained with these methods is problematic. Important to note is that the described saturation effect is assumed to be biased by several methodological conditions. The ISI and the design of stimuli presentation (randomised or in blocks) influences the occurrence of the paradoxical reduction (Näätänen & Picton, 1987). Moreover, the loudest tone presented to the subject is important, because an individual with a strong LDAEP might reach the point where the signal saturates earlier than one with a weaker LDAEP (Hensch et al., 2006). In the present study, the intensity levels varied between 10 and 60 dB SL (i.e. 30 and 80 dB SPL attested by audiometric measurements) and as such were relatively low. Nevertheless, the MEG responses to the lowest and highest tones were surprisingly distinct.

Finally, a number of important limitations need to be considered. One basic limitation of the present study is the fact that MEG is limited in its sensitivity for radially oriented sources (Ahlfors et al., 2010). On account of that, the extent of activated brain regions found during the N1/P2 time window could be restricted. A recent study from our group used simultaneous EEG and fMRI recordings to elucidate the underlying generators of the LDAEP (Neuner et al., 2014). In contrast to our study, additional activation in the insular cortices with high intensity tones was found here. Because the insular cortices are mainly radially oriented, it is likely that no signal was detectable. Furthermore, the determination of the auditory threshold was complicated by the background noise in the MEG scanner. Audiometric measurements revealed that the white noise level was similar to individual thresholds. Fortunately, human brains are specialized to filter out important information by means of attention and various acoustic cues (Ebata, 2003) leading to a distinct electrophysiological response.

6.3 Future directions

The present work adds important knowledge in the course of advancing the validation of the LDAEP as a potential biomarker. By means of referring to dimensional measures of symptoms in schizophre-

nia (e.g. severity of negative symptoms), the underlying neuronal dysfunctions can be identified more reliably than when referring to the phenotypic definition (see chapter 5.1). Additionally, study II showed that several other neuronal sources apart from the auditory cortex are involved in loudness processing (see chapter 5.2). This information should be included in the analysis of the LDAEP.

These findings suggest several courses of action for establishing rigorous and standardized protocols not only for the acquisition parameters of the LDAEP but also for its analysis. Discrepancies found throughout the research literature suggest that numerous variables have a marked influence on the results and impair the sensitivity and specificity of the LDAEP for 5-HT function (see chapter 2.3).

Basically, this easily implemented EEG-based method could be more largely used in practical clinical work to support clinicians in complementing their impressions and intuition with a more sophisticated approach (Juckel, Pogarell, et al., 2007). However, further research has to account for challenges bridging the science-to-service gap in psychiatry in order to advance the implementation of this paradigm in routine clinical practice.

A fruitful avenue would be the combination of different measures either to correlate the same construct as the LDAEP (e.g. measurement of 5-HT release capacity using PET (Quednow et al., 2012); serum BDNF levels (U. E. Lang, Hellweg, & Gallinat, 2005; Park, Lee, Um, & Kim, 2014)) or using measures that are linked to the construct (e.g. SSRI response prediction by theta activity in anterior cingulate cortex (Pizzagalli et al., 2001)). Furthermore, the specificity and sensitivity in individual treatment decisions or diagnostic accuracy of a psychiatric disease could be enhanced by the combination of several independent biomarkers in a multivariate analysis approach (e.g. pattern recognition) (Linden, 2012). The basic idea behind this approach is to use the combination of different parameters, as for example brain activity in different ROIs or connectivity measures, as well as imaging functional polymorphisms to separate patients from healthy individuals or to differ between diagnostic categories (see Klöppel et al. (2012) or Orrù, Pettersson-Yeo, Marquand, Satori, & Mechelli (2012) for a review).

A further way of improving the validity of biomarker findings in psychiatry lies in identifying the multiple genetic variants that contribute to the clinical phenotypes. As outlined in the introduction section (chapter 1.1), the LDAEP fulfils the requirements to stand as an endophenotype. However, since genetic association studies indicated that the LDAEP may also underlie influences by different neurotransmitter systems, the LDAEP's sensitivity and specificity for serotonin has been challenged (Guille et al., 2008; Massey, Marsh, & McAllister-Williams, 2004; Nathan, Segrave, Phan, O'Neill, & Croft, 2006; Norra et al., 2008; Proietti-Cecchini, Afra, & Schoenen, 1997; Segrave et al., 2006; Uhl et al., 2006). Associations with the dopaminergic system by means of the COMT polymorphism (Juckel, Kawohl, et al., 2008) or by dopamine transporter availability (DAT) (I. H. Lee et al., 2011;

Pogarell et al., 2004) were reported. Interestingly, short-time manipulations of the dopaminergic system as reflected in dopamine depletion (O'Neill, Guille, et al., 2008) and acute dopamine challenges with either pergolide, bromocriptine (O'Neill et al., 2006) or levodopa (Hitz et al., 2011) had no effects on the LDAEP. It is therefore assumed to be only manipulated by trait factors, i.e. genetic predisposition or chronic modulation of monoaminergic activity (O'Neill, Croft, et al., 2008; Simmons et al., 2011). Moreover, manipulating effects of glycine, a modulator of NMDA receptors, on the LDAEP have been found (O'Neill et al., 2007). Furthermore the gaseous molecule with neurotransmitter properties nitric oxide (NO) is thought to influence serotonergic-dopaminergic transmission. Kawohl, Giegling et al. (2008) found that functional single nucleotide polymorphisms (SNPs) of *NOS1* and *NOS3* were associated with the LDAEP.

In addition to possible neuromodulation by monoaminergic systems as discussed above these evoked potentials are also modulated by the phasic release of excitatory (i.e. glutamate) and inhibitory (i.e. GABA) neurotransmitter systems in the cortex (O'Neill, 2008; Simpson & Knight, 1993). Apparently, there is no clear evidence about the interaction of GABAergic neurotransmission and the generation of ERPs. In particular, no research has been done on LDAEP and GABA neurotransmitter activity (Kenemans & Kähkönen, 2011). Notwithstanding, a recent study reported high neural densities of GABA in the primary sensory and motor areas (La Fougere et al., 2011). Therefore, the relationship between LDAEP and GABAergic neurotransmission warrants closer investigation.

We intended to overcome these shortcomings by conducting studies that investigate the LDAEP and GABAergic neurotransmission in healthy humans. In the first study, we combined EEG with magnetic resonance spectroscopy (MRS) to measure the amount of free GABA and examined the relationship between this neurotransmitter and the neural processing of auditory stimuli in EEG. MRS is a very sensitive, non-invasive MRI technique, which allows the in vivo detection of endogenous metabolites in the human brain. However, we did not find any significant correlation between the amount of GABA in the auditory cortex and the LDAEP. Moreover, the variances of the effects were too high to guarantee a sufficient statistical power. There is evidence that the evoked gamma band response is highly correlated with resting GABA concentration (Muthukumaraswamy, Edden, Jones, Swettenham, & Singh, 2009) and in addition is modulated by sound intensity (Schadow et al., 2007). Therefore, the association between GABA levels and gamma-band oscillatory activity in the auditory cortex is currently under investigation. To our knowledge, this is the first study that measured GABA in the auditory cortex by means of MRS. In a second study we aim to investigate the LDAEP and GABAergic neurotransmission in healthy humans simultaneously. We therefore planned a study in a hybrid 3T MR-PET scanner (Herzog et al., 2011) with simultaneous EEG measurements that will be conducted during the next months. By using radiopharmaceutical compounds, for instance [11C]-flumazenil, PET can reveal the distribution of $GABA_A$ receptors in

human brain (Miederer et al., 2009). The combination of EEG, PET and MRI imaging techniques provides the unique opportunity to simultaneously measuring molecular and functional parameters that vary over time.

6.4 Conclusion

The present work extends previous research investigating the neurochemical aetiology of negative symptoms in schizophrenia by means of LDAEP. Our results revealed that serotonergic activity might be reduced in patients with predominantly negative symptoms. This is in accordance with medication strategies using atypical neuroleptic medication and antidepressants to enhance serotonergic neurotransmission in this subgroup of patients (Leucht et al., 2011; Silver, 2004). The LDAEP is therefore a valid instrument to clarify the underlying pathologies of symptom clusters in a heterogeneous diagnosis, such as schizophrenia, and may support individual treatment decisions. However, methodological factors which may have influenced the results must be considered and need further investigation. In particular the analysis strategies used for the LDAEP must be noted. Several studies confirmed that dipole source analysis and single electrode estimation lead to different findings and hence are not comparable (Hagenmuller et al., 2011; Jaworska et al., 2013). We therefore used source localization procedures by means of magnetic field tomography in MEG in order to provide a better estimation of the underlying sources involved in the generation of the LDAEP. Interestingly, the analysis showed that along with the auditory cortex additional brain areas are involved. Moreover, some of these areas such as the PCC are modified by serotonergic neurotransmission. This should also have an impact on the most promising utility of the LDAEP as a response marker for antidepressant treatment. Future research should employ standardized procedures in the acquisition and analysis of the LDAEP in order to ensure comparability between studies and to improve the validity of its qualities as a biomarker (Luck et al., 2011).

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Appendix

Table 1: Associations between genetic markers (*COMT*) and LDAEP mean values in left and right hemisphere across groups.

Hemisphere	Group	Mean	95% CI	Wald χ^2 (df)	Sig
Left	A-allele non-carriers (N=2)	1.612	1.363-1.908	6.746 (1)	.009 *
	A-allele carriers (N=13)	1.148	0.947-1.393		
Right	A-allele non-carriers (N=2)	1.413	1.168-1.711	3.697 (1)	.055
	A-allele carriers (N=13)	1.113	0.957-1.294		
Left	AA genotype (N=5)	1.286	1.023-1.617	7.423 (2)	.024 *
	AG genotype (N=8) ⎫ *	1.062	0.805-1.401		
	GG genotype (N=2) ⎭ *	1.612	1.363-1.908		
Right	AA genotype (N=5)	1.211	1.087-1.347	3.584 (2)	.158
	AG genotype (N=8)	1.052	0.828-1.337		
	GG genotype (N=2)	1.413	1.168-1.711		

Abbreviations: * $P < 0.05$.

Table 2: Associations between genetic markers (*SERT*) and LDAEP mean values in left and right hemisphere across groups.

Hemisphere	Group	Mean	95% CI	Wald χ^2 (df)	Sig
Left	S-allele carriers (N=2)	1.180	0.969-1.436	1.044 (1)	.307
	L-allele carriers (N=13)	1.409	1.066-1.862		
Right	S-allele carriers (N=2)	1.198	1.044-1.375	3.553 (1)	.059
	L-allele carriers (N=13)	0.861	0.629 -1.179		
Left	SS+SL genotypes (N=13)	1.180	0.969-1.436	1.044 (1)	.307
	LL genotype (N=2)	1.409	1.066-1.862		
Right	SS+SL genotypes (N=13)	1.198	1.044-1.375	3.553 (1)	.059
	LL genotype (N=2)	0.861	0.629-1.179		

Abbreviations: S, short variant; L, long variant.

Curriculum vitae

PERSONAL DETAILS

Name	Christine Wyss
Date of birth	September 22, 1979
Place of Birth	Männedorf
Nationality	Swiss

EDUCATION

11/2012-10/2013	Visiting Research Fellow Forschungszentrum Julich, Institute of Neuroscience and Medicine
05/2012-09/2014	PhD candidate in Psychology University of Zurich, Institute of Psychology, Division of Neuropsychology
10/2004-03/2010	Master of Science (MSc) in Psychology, Psychopathology and Neuroinformatics University of Zurich, Institute of Psychology, Division of Neuropsychology
08/2000-07/2002	General qualification for university entrance Cantonal graduate school for adults, KME Zurich
07/1996-07/1999	Federal Certificate of competence in Business Administration Zürichsee Medien AG, Stäfa

GRANTS AND FELLOWSHIPS

01/2013-01/2014	Mobility fellowship for doctoral students from the Swiss National Science Foundation (SNSF)
09/2013	Travel grant from the University of Zurich, Doctoral Program Psychology
07/2013	Travel grant from Schweizerische Akademie der Geistes- und Sozialwissen-schaften

PROFESSIONAL EXPERIENCE

05/2014-present	Research associate, European Union Seventh Framework Programme: TRIMAGE – a dedicated trimodality (PET/MR/EEG) imaging tool for

	schizophrenia, University Hospital of Psychiatry Zurich
04/2010-10/2012	Research associate, Program for Sustainable Development of Mental Health Services, Early Recognition and Intervention Program for Psychosis and Bipolar Disorder (ZInEP), University Hospital of Psychiatry Zurich
02/2009-04/2012	Executive assistant of the medical director Prof. Dr. W. Rössler, University Hospital of Psychiatry Zurich
04/2010-10/2011	Research associate, University Hospital of Psychiatry Zurich in collaboration with the Collegium Helveticum, Zurich
05/2008-02/2009	Job Coach, Department of Supported Employment, University Hospital of Psychiatry Zurich
09/2007-01/2008	Clinical internship, Department of Supported Employment, University Hospital of Psychiatry Zurich
10/2004-04/2008	Co-organizer of the Antiquarian Book Fair, EOS Buchantiquariat Benz, Zurich
10/2002-08/2004	Assistant of the deputy manager, Infel corporate media, Zurich

PEER-REVIEWED PUBLICATIONS

Wyss, C., Boers, F., Arrubla, J., Dammers, J., Kawohl, W., Neuner, I., & Shah, N.J. (2013). Loudness dependence of the auditory evoked N1/P2 component: A magnetoencephalography study. Neuroimage, 102P2, 465-473.

Neuner, I., Kawohl, W., Arrubla, J., Warbrick, T., Wyss, C., Hitz, K., Boers, F., Shah, N.J. (2014). Cortical signal variation in the processing of rising sound pressure levels: a combined event-related potentials and fMRI study. PloS One, 9(10), e109216.

Metzler, S., Dvorsky, D., Wyss, C., Müller, M., Gerstenberg, M., Traber-Walker, N., Walitza, S., Theodoridou, A., Rössler, W., Heekeren, K. (2014). Changes in neurocognitive functioning during transition to manifest disease: Comparison of individuals at risk for schizophrenic and bipolar affective psychoses. Psychological Medicine. In press.

Metzler, S., Dvorsky, D., Wyss, C., Müller, M., Traber-Walker, N., Walitza, S., Theodoridou, A., Rössler, W., Heekeren, K. (2014). Neurocognitive profiles in help-seeking individuals: comparison of risk for psychosis and bipolar disorder criteria. Psychological Medicine 44(16), 3543-3555.

Wyss, C., Hitz, K., Hengartner, M.P., Theodoridou, A., Obermann, C., Uhl, I., Roser, P., Gruenblatt, E., Juckel, G., & Kawohl, W. (2013). The loudness dependence of auditory

evoked potentials (LDAEP) as an indicator of serotonergic dysfunction in negative symptoms in schizophrenia. PLoS One, 8(7).

Hitz, K., Wyss, C., Hengartner, M.P., Kawohl, W. Associations of the endophenotype LDAEP and genetic polymorphisms of different neurotransmitters/neurochemicals, a review of the findings. In preparation.

Wyss, C., Tse, D. H.Y., Kometer, M., Arrubla, J., Kawohl, W., Neuner, I., Shah, N.J. Brain metabolism and its role in gamma-band oscillatory activity during auditory processing. In preparation.

CONFERENCE PRESENTATIONS

Wyss, C., Boers, F., Kawohl, W., Arrubla, J., Vahedipour, K., Dammers, J., Neuner, I., Shah, N.J. (2014). Neurobiological underpinnings of auditory intensity processing: a MEG study. Poster at the 1th Burghölzli Psychiatry Meeting, Zurich, Switzerland.

Wyss, C., Boers, F., Kawohl, W., Arrubla, J., Vahedipour, K., Dammers, J., Neuner, I., Shah, N.J. (2014). Understanding human neurobiological underpinnings of loudness perception: a MEG study. Poster at the 5th International Conference on Auditory Cortex, Magdeburg, Germany.

Metzler, S., Dvorsky, D.N., Wyss, C., Müller, M., Traber-Walker, N., Walitza, S., Theodoridou, A., Rössler, W., Heekeren, K. (2014). The course of neurocognitive functioning in helpseeking individuals: comparison of risk for psychosis and bipolar disorder criteria. Poster at the 4th Biennial Schizophrenia International Research Conference Meeting (SIRS), Florence, Italia.

Dvorsky, D.N, Metzler, S., Müller, M., Wyss, C., Heekeren, K., Walitza, S., Rössler, W., Theodoridou, A. (2014). At-risk states in psychosis: schizophrenia proneness instrument (SPI-A/SPICY) and its neuropsychological correlates. Poster at the 4th Biennial Schizophrenia International Research Conference Meeting (SIRS), Florence, Italia.

Wyss, C., Boers, F., Arrubla, J., Dammers, J., Kawohl, W., Neuner, I., & Shah, N.J. (2013). Loudness dependence of the auditory evoked N1/P2 component: A magnetoencephalography study. Poster at the International Conference on Basic and Clinical Multimodal Imaging, Genève, Switzerland.

Tse, D.H.Y., Wyss, C., Arrubla, J., Kawohl, W., Neuner, I., Shah, N.J. (2013). GABA detection in primary auditory cortex by magnetic resonance spectroscopy without spectral editing. E-poster at the 30th Annual Scientific Meeting of European Society for Magnetic Responance in Medicine and Biology (ESMRMB), Toulouse, France.

Wyss, C., Hitz, K., Hengartner, M.P., Theodoridou, A., Obermann, C., Uhl, I., Roser, P., Gruenblatt, E., Juckel, G., Kawohl, W. (2013). Evoked potentials as indicators of serotonergic dysfunction of negative symptoms in schizophrenia. Poster at the annual Meeting of the Organization for Human Brain Mapping (OHBM), Seattle, USA.

Wyss, C., Hitz, K., Hengartner, M.P., Theodoridou, A., Juckel, G., Kawohl, W. (2012). Serotonerge Aktivität und Negativsymptome der Schizophrenie. Poster at the Tag der Forschung an der Psychiatrischen Unversitätsklinik Zurich, Switzerland.

Wyss, C. (2010). Wie erkenne ich die Frühform einer Psychose? Workshop at the 20th Interaktiver Hausärztenachmittag at the University Hospital Zurich, Switzerland.